mitchell (S. Weir) 28
+ Reichert (Ed. J.)

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PRELIMINARY REPORT

ON THE

VENOMS OF SERPENTS.

BY

S. WEIR MITCHELL, M.D.,

AND

EDWARD T. REICHERT, M.D.,

OF PHILADELPHIA.

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PRELIMINARY REPORT ON THE VENOMS OF SERPENTS.

THE present report represents a part only of an elaborate study of the poisons of all our own genera of serpents, and it is hoped may include a number of foreign genera. Our researches have of late been rewarded by so remarkable a discovery in toxicology that it has been thought well to announce it here rather than to await their completion. We have therefore selected from our notes such material as seems to us of interest from its novelty.

Physical Characters.—The venoms which we have obtained in a fresh state from the rattlesnake, moccasin, and copper-head, do not differ in general appearances, and are all in the form of a slightly turbid, yellowish fluid (occasionally colorless), and varying more or less in the degree of viscidity. They have no odor, and the reaction is invariably acid.

All the venoms, whether in their dried or their natural state, are soluble in water at ordinary temperatures, save for a slight cloudiness which but slowly settles. In the dry cobra venom sent us from India, by Mr. Vincent Richards, of Calcutta, there is also a little indissoluble matter, which

occurs in larger flakes, and may be due to the mode of drying the poison.

When dried they resemble ordinary egg-albumen, and when thus prepared in small quantities in a porcelain capsule, innumerable radiating lines of fracture occur which break the mass into long, needle-like pieces, closely resembling acicular crystals; indeed, the resemblance is so striking that the uninitiated are frequently deceived as to the true physical condition of the venom. When larger quantities of venom are dried, these needle-like pieces are apt to be broken up by numerous cross-fractures.

In drying, venom loses a large proportion of its weight. Unfortunately, we have not had at our disposal any fresh cobra venom, nor have we as yet made any estimate of the solid constituents of the venom of the copper-head, but in our examination of the venoms of the rattlesnake and moccasin, we found a loss of nearly seventy-five per cent.

Aqueous solutions still preserve the acid reaction, and, apparently, all the properties of the fresh venom, excepting that the intensity of the poison is somewhat diminished.

These venoms, or their solutions, can be subjected to the boiling temperature of water (except the venom of the *Crotalus adamanteus*) without a complete destruction of their poisonous power, but with a noticeable alteration of their physiological properties. In the case of the *Crotalus adamanteus*, or the *diamond-back rattlesnake*, the toxicity of the venom is destroyed at a temperature below 80° C.

(176° F.). As yet we have not had the opportunity of studying the venom from the *Crotalus durissus*, or other species of rattlesnakes, but one of us (Mitchell) determined years ago that the poisoning power of the venom of the *durissus* is not destroyed at a boiling temperature. It certainly is a very curious fact that the venom of the *adamanteus* is so different from other venoms, in being destroyed at so low a temperature, and our knowledge at present is not such as to offer any satisfactory explanation.

The external symptoms caused in animals by these several venoms—cobra, rattlesnake, moccasin, or copper-head—do not differ radically, save in degree. In all alike there is some primary heart disturbance, temporarily lowered blood-pressure, fatal enfeeblement of the respiratory centres, and local effusion of blood with lessening or loss of its power to clot. These latter symptoms are best seen when the animal survives for some hours or a day, and then also is noticeable the breaking-down of the capillaries and the tendency to local putrescence and gangrene.

There are, however, certain symptoms which make it probable that, as our studies already indicate, we shall be enabled, after further investigation, to point out certain differences which may make it possible to discriminate any one form of poisoning from the others. Beyond a doubt, cobra venom is the most intense in its poisonous power, the venom of the copper-head next, then the moccasin and rattlesnake. Our investigations

in this line are as yet far from complete, and we accordingly do not assert these facts as being final or at all conclusive.

The statement of Gautier, of Paris, that he had found an alkaloid in cobra poison resembling a ptomaine, has not received any support from what we have done in the chemistry of venoms; nor has Prof. Wolcott Gibbs been, so far, any more fortunate in finding an alkaloid in the *Crotalus* poison with which we supplied him. Our work has however resulted in the isolation of three distinct proteid bodies, of which two are soluble in distilled water, and one is not. Of the former two, one is incoagulable at a temperature of 100° C. It may be obtained by boiling venom, which throws down or destroys all the other proteids, and then filtering, or by dialysis.

The fact that this proteid remains uncoagulated by boiling and will dialyze, renders it certain that it belongs to a peculiar class of bodies which are ordinarily the result of peptic or tryptic digestion, and are known as peptones. In order to determine more definitely its character we prepared a solution of *moccasin* venom by the first process, and subjected it to a careful series of tests, designed to determine more exactly its place in the family of proteids, and with these results:

(1) Readily dialyzable.

(2) Not coagulated at a temperature of 100° C.

(3) Reaction with the xantho-proteic test (nitric acid and ammonia).

(4) Reaction with Millon's reagent (mercuric nitrate).

(5) No precipitate with weak or strong nitric acid.

(6) No precipitate with CO_2.

(7) No precipitate with ferric chloride.

(8) No precipitate with cupric sulphate.

(9) Precipitated by mercuric chloride.

(10) Precipitated by absolute alcohol.

(11) Gives a faint reddish tinge with a strong solution of potassium hydrate, and a trace of cupric sulphate.

(12) Not precipitated by strong acetic acid (glacial).

(13) Precipitated by very dilute acetic acid, precipitate being redissolved by further addition of acid.

(14) Full reaction with Adamkiewicz's test for peptones.[1]

(15) Precipitated by adding a large quantity of sodium chloride, the precipitate being redissolved on the addition of a large quantity of glacial acetic acid.

(16) Precipitated by mercuric nitrate.

(17) Precipitated by absolute alcohol, precipitate being apparently redissolved on the addition of water.

(18) Precipitated by saturation with potassium hydrate, precipitate being redissolved by the addition of nitric acid, with the formation of a decidedly yellow solution (xantho-proteic) which becomes decolorized by addition of acid.

(19) Precipitated by potassium ferrocyanide in the presence of weak acetic acid.

In a critical examination of these reactions it will be observed that while the peptone in question answers to these tests in such a positive manner as

[1] As this test is not to be found in the ordinary text-books on physiology or physiological chemistry, we will state that the test consists in first adding a little sodium chloride to the suspected mixture, then some strong acid acetic, followed by acid sulphuric. This gives a lake color turning to violet, and the top of the solution has an opalescent olive tinge.

to place the matter of its nature beyond doubt, there are certain reactions which are so novel as to give this body characteristics which will distinguish it from all others of its class. These peculiarities are to be found in Nos. 13, 15, and 18. We are not aware that there is any other peptone giving such reactions by these tests.

But what is much stranger is that the peptone we have discovered is the only one as yet known to constitute a portion of a secretion, or to originate within the living body in any way except as a product of the digestion of proteids.

Some of the solution of this proteid being allowed to dry at a temperature somewhat below 100° C., it was afterwards found impossible to completely dissolve it in distilled water, the mixture remaining full of coagula, which appear to be wholly insoluble. When the coagula are filtered off, it is found that the filtrate gives all the reactions as before, although the poison in it, of which we will make further mention, appears to have entirely lost its power; nor were we able to demonstrate the existence of any toxicity in the coagula. We, therefore, do not know whether after the boiling of *moccasin* venom there remain two proteids in solution or one, but the fact of the complete loss of toxic power, under the circumstances just described, indicates that the two proteids formed in the last instance result from the breaking up of the original peptone. It seems strange, however, that, if the original body be broken up, the peptone which still remains in solution should retain the chemical peculiarities of

the original substance, and answer to all the tests as we have carefully determined. The dialyzed peptone from the *Crotalus*, when dried at 40° C., entirely redissolved upon the addition of distilled water, and is poisonous, and also answers to all the above tests.

The second proteid separated by us from the original venom solutions is quite as interesting as the peptone.

We have already alluded to the fact of the occurence of a precipitate when fresh venom or its aqueous solution is allowed to stand for some hours undisturbed. The appearance of this precipitate gives one the impression of its being an albuminous body, and this only seems the more probable since the precipitate is entirely dissolved by the addition of a small amount of sodium chloride, very weak acids, or alkalies. But in order to determine more fully the nature of this substance, we prepared an aqueous solution of *Crotalus* venom and allowed it to stand for twenty-four hours. The supernatant liquid was decanted without disturbing the precipitate, which was then repeatedly washed with distilled water until the decanted liquid gave no reaction for albuminoids and chlorides. The precipitate thus prepared, and which, of course, is wholly insoluble in distilled water, gave the following reactions:

(1) Dissolving by the addition of a small amount of sodium chloride.

(2) *Partially* precipitated from weak solutions of

sodium chloride by addition of sodium chloride to saturation.

(3) Soluble in weak solutions of magnesium sulphate, but apparently *entirely* precipitated by addition of the salt to saturation.

(4) Precipitate soluble in weak acids, and again precipitated by addition of strong nitric acid.

(5) Gave proteid reaction with xantho-proteic test.

(6) Gave proteid reaction with Millon's reagent.

(7) Coagulated in weak neutral saline solutions at a temperature of about 68.5° C.

The precipitate obtained by the addition of magnesium sulphate (3) is flaky in character, contrasting with the fine precipitate deposited by saturation with the sodium chloride, and was much more abundant.

These reactions indicate without doubt that the precipitate is a proteid belonging to the *globulins*, and that it most resembles *paraglobulin*.

We have also obtained this substance during the process of the dialytic separation of the peptone, when the globulin precipitates within the dialyzer, and it may be separated by filtration or decantation and washings. The globulin principle obtained from *moccasin* venom differs from that obtained from the *Crotalus* in that (1) by boiling it is entirely dissolved in the water, instead of being coagulated; (2) that it requires much stronger alkaline and acid solutions to dissolve it.

After the extraction of the *peptone* and *globulin* principles, there still remains in solution a third body, which becomes turbid at a temperature of about 65.5° C, and coagulates a few degrees higher,

and which gives reaction with the xantho-proteic and Millon's tests for proteids, and is precipitated with weak alkalies or acids. We have not as yet been able to isolate this proteid free from contamination with the globulin and peptone principles, but judging from the fact of its perfect solubility in water, the point of coagulation and behavior with dilute alkalies and acids, it is an *albumen*. As ether does not precipitate it, it is more akin to *serum-albumen* than to egg-albumen. We are now endeavoring to obtain this principle pure by dialyzing the peptone off, and afterwards separating the globulin principle from it by filtration, when we hope to be able to study its properties with more definiteness.

The whole subject of the chemistry of proteids is as yet in such an uncertain and extremely unsatisfactory condition that the best we can do now is simply to indicate to which general class of proteids these several bodies belong, and in order to distinguish them, we propose therefore for the present to call them *venom-peptone*, *venom-globulin*, and *venom-albumen*.

Our study of the physiological properties of this *venom-peptone*, though incomplete, clearly shows that while it is poisonous, it is far from possessing all the poisonous features of venom. It is much slower in its action than venom, and the local effects produced by it are of an œdematous character, contrasting strongly with the action of the pure venom, which almost immediately causes great darkening of the tissues, due to infiltration of blood which is wholly or at least partially

incoagulable. When the venom-peptone is injected into the breast muscles of a pigeon, if the animal dies within an hour or so, there is scarcely any appreciable local effect, but if the dose has been smaller, the first local effect observed is a considerable œdematous swelling in the form of an abruptly protruding lump, but without any dark discoloration. At the end of twenty-four or thirty-six hours some slight discoloration is at times observed beneath the skin, and after forty-eight hours there is a discharge from the swelling of a putrescent, muddy-looking serum. If the muscles of the side are now cut into, they will be found to be considerably congested, to be marked with greenish streaks, and to give off horrible putrefactive odors. In a pigeon, killed before the end of twenty-four hours, we found beneath the œdematous swelling a cavity about an inch in diameter, which was full of broken-down tissue, having a muddy, gangrenous appearance, and a putrefactive odor, while the surrounding muscular tissues were normal in appearance. Judging from the fact that the venom-peptone does not give rise to any darkening of the muscular tissues within a short while after injection, and, indeed, as it seems probable, not until putrefaction has set in, it seems to us likely that the darkening and congestion which ultimately occur, are to be regarded as mere secondary effects, and due to the putrefactive changes induced by the poison. The remarkable power possessed by this poison to cause putrefaction in the living tissue in so short a time is astonishing, and will exact a long and patient investigation.

These differences between the effects of the *venom-peptone* and pure venom lead us to believe that the former is not the only poisonous principle in venom, and that it is the least important in point of activity. We have been so fortunate as to find a second poison factor, and one of intense activity, in *venom-globulin*. This poison is of such virulency that one-twentieth of a grain is sufficient to kill a strong pigeon in a little over two hours, and to give rise within a few minutes after injection to the production of enormous infiltration of blood into the neighboring tissues.

In two experiments made on rabbits to determine the actions of these two principles on the blood-pressure, we found that the *venom-peptone* caused an immediate fall of blood-pressure to about one-half of the normal, after which convulsions came on accompanied by a rise of pressure to above the normal. The injection of several more doses did not affect the pressure as did the first dose—these results corresponding to what we have observed in experiments with the fresh venom. The *venom-globulin* poison seems to be devoid of any action on the blood-pressure, except to make more prominent the so-called vaso motor curves. We also noticed in these experiments that the *venom-globulin* gave rise to bloody extravasation in the peritoneum, such as is observed in venom poisoning, whilst the *venom-peptone* did not.

As yet we have not satisfied ourselves as to whether *venom-albumen* is a third poisonous factor, since we have been unable to isolate it in a condition in

which we were satisfied as to its absolute purity, on account of our having been baffled so far in completely getting rid of the venom-globulin. The results, however, obtained from our very incomplete study of the physiological properties of the *peptone* and *globulin* principles indicate that they fully represent all the poisonous qualities of venom, the *venom-globulin* being decidedly the more poisonous of the two, and, undoubtedly, the essential poisonous element.

Up to this date, all observers have regarded the venoms as representing a single poison. We have been able to show that the venom of the *moccasin* and *C. adamanteus* contains three proteids—one analogous to peptones, and a putrefacient; one akin to globulins, and a much more fatal poison, probably attacking the respiratory centres, and destroying the power of the blood to clot; and a third resembling the albumens, and probable innocent.

Finally, we have learned that the poisons of the rattlesnake (*C. adamanteus*), copper-head (*Agkistrodon contortrix*), and moccasin (*Toxicophis piscivorus*) are capable of being destroyed by bromine, iodine, bromohydric acid (thirty-three per cent.), sodium hydrate, potassium hydrate, and, as Lacerda has shown, by potassium permanganate.

The separation of the two poisons necessitates, of course, a long and elaborate series of researches, the results of which we hope to report in future.

Laboratory of Physiology,
University of Pennsylvania.