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The Action of Rattlesnake Venom

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Bactericidal Power of the Blood Serum.

BY

CHARLES B. EWING, M. D.,

CAPTAIN AND ASSISTANT SURGEON, UNITED STATES ARMY.

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THE ACTION OF RATTLESNAKE VENOM UPON THE BACTERICIDAL POWER OF THE BLOOD SERUM.[1]

BY CHARLES B. EWING, M. D.,
Medical Department, United States Army.

An apology would be necessary for introducing a subject of this character, on the present occasion, if it were not that the loss of life from poisonous reptiles has become so great that it calls forth the best effort of not only the military surgeon but of medical men generally, to lessen it.

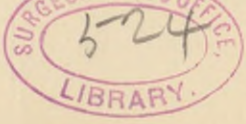
The mortality in India alone reaches the alarming figure of 20,000 persons annually; hence it was not a strange coincidence that it should have fallen to the lot of the British Medical Service, led by Dr. (now Sir) Joseph Fayrer, to perform the pioneer work in that part of the globe.

While much has been written upon this subject in general, very little has been said or done in the special line to which this paper relates.

At Professor Welch's suggestion, we conducted a series of experiments in the pathological laboratory of the Johns Hopkins University, during the spring of 1893, having for their purpose the determination of the action of rattlesnake venom upon the bactericidal power of the blood.

Dr. Welch's attention was directed to the investigation of this subject by Dr. Weir Mitchell. Drs. Mit-

[1] From the proceedings of the Washington meeting of military surgeons.



chell and Reichert demonstrated that the poisonous properties of rattlesnake venom depend upon the presence of proteid substances. These investigators were the first to demonstrate the existence of the so-called toxic albumins.

In their monograph on the subject, as well as from previous observations, it was apparent that the animals killed by rattlesnake venom decomposed with great rapidity—indeed with such rapidity that Dr. Formad, who contributed an appendix upon the pathological anatomy of animals dead of rattlesnake venom, believed that there was evidence of the spontaneous generation of bacteria.

Dr. Formad thought it impossible for the bacteria to make their way into the circulation and multiply so quickly. This seemed so improbable to Professor Welch that he suggested to Dr. Weir Mitchell the importance of having this point worked up. Dr. Mitchell kindly gave us a certain amount of poison. We were, however, fortunate enough to obtain a live rattlesnake of the Diamond species, known as the *Crotalus Adamanteus*, from which fresh venom was obtained and used in preference to the dried poison supplied us.

It will not be out of place to recall briefly, that the order *Ophidia*, of the sub-class *Reptilia*, is divided into three subdivisions: (1) the *Ophidii Colubriformis*, or innocuous snakes; (2) the *Ophidii Colubriformis Venenosi* or poisonous colubrine snakes; (3) the *Ophidii Viperiformes*, or viperine snakes, poisonus.

To the last sub-division, viperine snakes, belong the *Crotilidæ*, or rattlesnakes, called by Fayrer "pit-viper," being distinguished from the *Viperidæ* or vipers proper, by possessing a pit or depression between the

eye and the nostril in the loreal region; a triangular broad head and thick body of variable length.

The crotalus is distributed widely over the globe, and all are terrestrial except in India, where quite a number of genera are arboreal; these, however, lack all semblance to the horny scales or rings at the tip of the tail, known as the "rattle," except in the species called *Halys*, where the tail terminates in merely a rudimentary horny spine.

It is of interest to note that the Indian crotilidiæ are not so venomous as their American congeners, though Asia has a very deadly species known as the *Calloselasma Rhodostoma*, resident in Java and Siam while South America is represented by the well-known *Jararacca* or *Craspedocephalus Braziliensis*. Australia and Africa likewise contain ophidians of this class, but they are little known.

The crotilidæ of the United States are represented by ten species and three sub-species, all being equally poisonous so far as we now know. Of these the *Adamanteus* has three sub-species, one of them, the *Adamanteus* proper, found in this country, extending from North Carolina to Florida; a second, the *Atrox*, resident in the Indian Territory and Texas, also throughout that stretch of country extending to Sonora and southern and lower California; and a third called *Scultulatus*, known only to Arizona.

We had in view in the first place to test the question as to whether the blood of animals killed by rattlesnake poison had lost any of its germicidal power.

We used Dr. Weir Mitchell's noose for securing the snake, and with a sterilized saucer thrust into the mouth of the animal, we collected the venom. In this way

we had no difficulty in getting from 0.5 to 1 c. c. of nearly clear, slightly straw-colored fluid, quite sufficient for our purposes. This was diluted with an equal quantity of sterilized physiological salt solution, and 0.25 to 0.5 c. c. of this mixture was inoculated subcutaneously, under antiseptic precautions, the dose varying according to the time we wished the animal to live. This was usually from one-half to one hour and a half, but in one or two cases, three hours after the injection.

The injections were in all cases given subcutaneously, sometimes beneath the skin of the abdomen and at other times in the groin. The lesions differed somewhat, according to the site of injection. When made beneath the skin of the abdomen, there were most extensive peritoneal hemorrhages, whereas, when the injections were made in the thigh, these lesions were less extensive although not absent; here the most striking effects noted, beside the deeply discolored integument surrounding the point of injection, were the underlying hæmorrhages into the muscles.

The important lesions were these: Most extensive hemorrhage, with disintegration of tissue and actual necrosis of tissue, for a wide distance around the point of inoculation, with ecchymosis in other parts of the body, particularly in the serous membranes. Another point which served our purpose admirably was that the blood does not coagulate after death, or coagulates only feebly and after a long interval. We therefore had no difficulty in collecting a sufficient amount of the fluid blood, and we did so by withdrawing the blood in less than a minute after the animal breathed its last. We exposed the heart at once, and with sterilized in-

struments made an incision in the right auricle, and with a sterilized pipette we aspirated from the right heart; and then passing the pipette down into the abdominal vena-cava, we secured sometimes as much as seven or eight cubic centimetres of blood. This blood was collected in a sterilized test-tube and put in the refrigerator. After twenty-four hours, the red blood corpuscles had settled; sometimes a small, soft, dark coagulum had formed, and there was a layer of clear serum.

The usual statement is that the blood does not coagulate. It looks as if it did not coagulate, and as though there was merely a sedimentation. But there is in fact an extremely thin clot, which does not retract from the sides of the test-tube. It is an extremely viscid, stickly coagulum, which adheres to the platinum needle inserted into it. We pipetted off after twenty-four hours the clear serum, usually collecting 0.5 to 1.5 c. c. The organisms which were used to test the germicidal power of the serum were the bacillus coli communis and the bacillus anthracis. Professor Welch selected the bacillus coli communis because that is an organism which is normally present in the intestine, and also one which he had experimented upon with normal serum He took the bacillus anthracis because that is a classic organism in regard to the action of serum. The results were uniform and striking. As a rule we made a control experiment with normal serum; thus, we killed a healthy rabbit and withdrew the serum in the same way, collecting the same quantity, and inoculated at the same time a parallel control, tubes with the bacteria to be tested The cultures were twenty-four to forty-eight hour cultures. Our bacillus

anthracis was obtained in suspension from the spleen of an animal recently dead of anthrax. We wished to obtain the anthrax bacillus free from spores, and thought it better to take it fresh from the animal. Similar results were obtained also with twenty-four-hour anthrax cultures grown at room temperature. We made a suspension in salt solution of the organisms, and inoculated a known quantity, one to two platinum loops of the suspension, into the serum tube. Then we made roll cultures.

The following tables, drawn up by Professor Welch, represent only a certain number of our experiments, and are much abbreviated for the sake of clearness:

BACILLUS ANTHRACIS.

Normal Serum.

No.	No. bacilli inoculated.	After 24 hours.	After one week.
1 A	485	0	0
1 B	8,512	0	0
1 C	210	0	0
2 A	5,292	0	0
2 B	1,628	0	0

Venom Serum.

No.	No. bacilli inoculated.	After 19 hours	After 24 hours.
1 A	8,512	Countless.	
1 B	25	.....	3,233
2	68	.....	250,000
3	3,292	Countless.	
4	3,292	Countless.	
5	3,292	Countless.	

BACILLUS COLI COMMUNIS.

Normal Serum.

No.	No. Bacilli inoculated.	Immediately.	After 24 hours.	After 48 hours.	After one week.
1A	250,000	256	157	Countless	Countless
1B	175,000	188	50	Countless	Countless
2A	29,161	53	0	0	0
2B	3,150	18	0	0	0
3	9,990	13	38	625	Countless
4	84,947	238	0	0	0

Venom Serum.

No.	No. bacilli inoculated.	Immediately.	After 22 hours.
1	800,000	1,077	Countless
2	3,150	6	26,000
3	16,560	32	Countless
4	9,990	35	Countless
5	84,947	284	Countless
6	84,947	231	Countless
7	84,947	182	Countless

With the bacillus coli communis, it will be observed that in the case of normal serum, we inoculated 250,000 bacteria. Immediately afterward, there were 256 colonies; after twenty-four hours only 157 colonies. In other words, there was not only no development, but an actual diminution in the number of bacteria. If the organisms are not all killed at the end of twenty-four hours, the germicidal power ceases, and those which remain multiply. After forty-eight hours, the colonies were countless. In the second experiment we inoculated 175,000 bacteria, which represented 188 colonies immediately. After twenty-four hours there were only 30 colonies, and in forty-eight hours they were countless. Taking a smaller number of bacteria, 29,161, there were immediately 53 colonies; after twenty-four hours, none; after forty-eight hours, none; after one week, none. In other words, in twenty-four hours the normal serum had destroyed 29,000 bacteria.

These experiments were uniform in result and point to one conclusion, that the blood of rabbits killed in one-half to three hours after subcutaneous inoculation with rattlesnake venom, has lost its germicidal power. This is of considerable interest, for it is an indication of a very profound alteration in the blood. This germicidal power of the blood is one of very great significance, on which many of the modern theories of immunity depend.

It is of special interest to ascertain under what conditions the germicidal properties of the normal blood serum are at their highest, and in what way these properties affect the composition of the blood.

The principal workers in this field have been Von Fodor, Nuttall, Wasserman, Kitasato, Buchner, Ogata,

Hankin and others. Von Fodor's work had reference to the composition of the blood, and was intended to show that arterial has a more destructive action on bacteria than venous blood, and also that fresh blood has a more destructive action than that which has been standing for some time. It was also found that the germicidal power of the blood was weakened in an atmosphere of oxygen or carbonic acid gas, but the removal of gases from the blood had no appreciable effect.

A series of experiments showing the effect of moving and stationary blood upon bacteria by means of small globes containing blood, some of which were kept in constant motion and others quite stationary, resulted in no appreciable difference being observed.

Temperature affected very materially the bactericidal power of the blood, which increased with the rise of temperature from 38° to 40° C., and then gradually diminished with the fall.

Von Fodor is of the opinion that the individual predisposition of an animal to an infectious disease stands in close relationship with the germicidal power of the blood.

A second series related to the influence of drugs on the power of blood to destroy germs.

Hydrochloric acid had no effect; tartaric acid and quinine, respectively, produced a marked decrease. A slight increase was produced by common salt and carbonate of ammonium, but a more marked effect by the phosphate of sodium, while the carbonate of potassium and sodium showed a very remarkable increase. From these experiments the deduction was made that the bactericidal power of the organism was raised by any

drug which increased the alkalinity of the blood. The third series verified the conclusions regarding the alkalization of the blood. Of eight rabbits inoculated with anthrax all died, whilst of nineteen which had been previously injected with a solution of soda, only three died. A majority of the sixteen remaining were perfectly free from disease, only a few being fatally affected.

Up to this time, however, the doctrine of phagocytosis as advanced by Metchnikoff held sway, when Nuttall struck the first severe blow to this theory. He, in his most excellent inaugural dissertation at Göttingen, in 1890, showed that the destruction of virulent bacteria in the blood of animals by the leucocytes was not at all essential, but that the serum of blood free from all cellular elements possessed this power to a degree equal to the blood in its entirety.

Nuttall's work is graphically shown in some twenty-eight tables in his "Beitrage zur Kenntniss der Immunitat." He here proves very conclusively that in the blood, bacteria presented marked evidences of degeneration before being taken up by the wandering cells, or luceocytes. It was also seen that the bactericidal power of the blood of different animals varied, and that while in some, certain bacteria were promptly killed, in others these were simply restrained for a time, or not affected.

Buchner, Lubarsch, Nissen, Stern and Prudden have practically verified these observations. Buchner, particularly, in his experiments upon dogs and rabbits, verified the findings of Nuttall, but went even further, and proved that the bactericidal power of the blood of these animals did not *at all depend* upon the cellular elements, but resided in the clear serum which sepa-

rate dfrom the clot, after the blood had stood a while in a cool place He also demonstrated that the germicidal action of blood and serum was destroyed by exposure for an hour to 55° C., or by heating to 52° C. for six hours, or to 45.6° C for twenty hours.

Alternate freezing and thawing did not destroy the bactericidal power of the serum, but it was diminished or completely checked by dialysis with distilled water, or by extreme dilution with the same. He preserved the anti-bacterial action of the serum by making an equal dilution with a six-tenths to seven-tenths per cent of sodium chloride solution, and was led to believe that the activity of the serum was greater alone, than when the cellular elements of the blood were present; hence he concluded that the active element is a living albumin, having as an essential constituent an alkaline base. This albuminoid substance is thought by Hankin to be identical with his "globulin," isolated from the spleen and lymphatic glands.

According to the views of these experimenters, the germicidal power of the blood resides in the serum alone, and phagocytosis is but a secondary process, the leucocytes taking up the bacteria only after they have been rendered inert by the germicidal power of the serum of the blood and certain other fluids of the body. For our purpose, however, it is not necessary to insist upon the humoral as opposed to the phagocytic doctrine of immunity. All that concerns us is the recognition of the bactericidal power of the blood serum under certain conditions.

The loss of this normal germicidal power helps us to explain the varying rapidity with which post-mortem decomposition sets in. It is well known that per-

sons dead of different diseases decompose with varying degrees of rapidity. We can not explain this differing rapidity of decomposition simply by variations in temperature, for under the same external conditions one body will be decomposed in a comparatively few hours, and another may remain undecomposed for several days, We selected the animals killed with rattlesnake venom because it is well known th t they decompose with great rapidity. The bodies of human beings killed by snake venom are also said to decompose with great rapidity.

The results of our experiments furnish a satisfactory explanation of this phenomenon. The blood at the time of death, and even before death, has lost all, or nearly all, power of resisting the invasion and multiplication of certain bacteria, so that the bacteria of putrefaction which are normally present in the intestine develop with astonishing rapidity, and even before the animal is cold, produce this wonderful rapid decomposition. Our experiments are also suggestive as regards certain secondary and mixed infections. The toxic proteids of snake venom belong to the same class of poisons as those formed by toxic bacteria, such as the bacillus of tetanus, of diphtheria, etc.

It is easy to suppose that these infectious diseases may cause a diminution of the germicidal power of the blood against sdcondary invaders, of which common examples are the pyogenic bacteria, present often in our mouths and intestinal canals, and which, in an individual whose resistance is lowered by a loss of the germicidal power of the blood, may grow and multiply. In other words, we can understand better the causation of many of these secondary infections.