CASE OF RATTLESNAKE BITE IN LONDON.

By SIR JOSEPH FAYRER, K.C.S.I., M.D., F.R.S.

ON August 4th a gentleman, of about 30 years of age, came to me at about 10 P.M. with his hand bandaged. He informed me that about an hour previously he had been bitten by a *crotalus durissus*, 4 feet long. The accident happened in this wise:

It appears that, being interested in poisonous snakes, he keeps several as pets. He was feeding this rattlesnake, and was pushing a dead mouse into its throat with his finger when the reptile imbedded one fang in the mouse and the other in his finger, near the apex and by the side of the nail. He withdrew his finger rapidly; it was bleeding freely. He sucked the wound, went immediately to consult a medical man, who most judiciously applied a ligature tightly round the finger, gave him some whisky, made an incision into the puncture and applied nitric acid to it. He then went to a hospital, where he received further attention and was invited to remain; this however he declined to do, and came on to my house.

When I saw him, about an hour after being bitten, he looked fairly well, not frightened, but rather confused and excited—perhaps from the whisky which he had taken. He said he had swallowed about a tumblerful of that stimulant. The finger was much swollen, probably from the ligature, and the wound discolored from the nitric acid. The ligature had been removed and the wound was still oozing blood; it had been carefully dressed at the hospital. There were neither constitutional indications of poisoning nor pain in the injured part. He said he had a tingling sensation in the mouth, but this was probably due to the whisky he had imbibed.

I re-applied the ligature tightly round the finger and sent him back to the hospital at once, to remain there all night under observation, giving him a note recommending that the ligature should be kept on as long as it safely might be, and that a solution of permanganate of potash, 1 to 10 or 20 should be injected into the wound, which should also be allowed to bleed freely if it would. I urged him to lose no time.

Next day, at 1.30 P.M., he called at my house when I was out; he did not appear to be suffering, I was told.

On August 6th I met him walking in the street; his hand was still bound up, but he had suffered no further inconvenience. He said he had spent the night in the hospital, and was allowed to leave on the following morning.

I heard nothing further of him till August 20th, when he presented himself in his usual state of good health, the wound on the finger still unhealed, but looking healthy; evidently there had been no constitutional poisoning, but the finger was still swollen.

The case is interesting, as it throws light on the explanation of those cases of bite from the more venomous snakes in which no evil result ensues.

In this instance the patient had been clearly punctured by the long vibratile fang of a large rattlesnake, irritated and hungry for its food. The bite was probably not intentional, and, the finger being rapidly withdrawn, no time was allowed for compression of the jaws or forcible injection of the poison into the puncture. It was, moreover, promptly and judiciously treated immediately after the bite, and this alone might account for the immunity from symptoms of poisoning. I am inclined, however, to believe that either no poison had been injected at all, or so little as to cause no ill effects. It is in this way that people do sometimes escape, and it is thus that so-called antidotes sometimes acquire a reputation they do not deserve.

It is to be hoped that this will prove a salutary lesson to this gentleman, and make him more careful in future how he handles and feeds his dangerous favourites; a little more pressure of the jaws, with a little deeper penetration of the fang, and a less happy result might have been recorded.

I need hardly say the case was full of interest, and it was a source of great satisfaction to find that both on the part of the patient and the medical adviser all that presence of mind and common sense, combined with appreciation of the urgency of the case could suggest, had been so promptly and vigorously done.

It is curious that I should have had two opportunities of seeing cases of snake bite in London, the first from a puff adder, *vipera arietans*; the second from a rattlesnake, *crotalus durissus*. In both cases the result was favourable.

REPORTS

TO THE

SCIENTIFIC GRANTS COMMITTEE

OF THE

BRITISH MEDICAL ASSOCIATION.

REPORT ON

THE BACTERICIDAL ACTION OF ALEXINS.

By E. H. HANKIN,

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[From the Pathological Laboratory, Cambridge.]

I HAVE elsewhere shown that proteid substances capable of killing bacteria, and now known as alexins, can be obtained from the bodies of various animals. Further, I have given reasons for thinking that during life these bodies are contained in cells; that by an appropriate stimulus on the death of the animal they may be liberated, and pass into the body fluids; that the bactericidal power of the cell-free blood serum (which has been ably dealt with by Buchner and others) is probably due to the presence of traces of these alexins. Lastly, I have shown that from the chemical standpoint these bodies are identical with Halliburton's cell globulin- β , a substance having the same action on blood plasma as fibrin ferment.¹

A belief is prevalent that the bodies of animals killed by a lightning flash putrefy with unusual rapidity, and that their blood refuses to clot. If this is so, we might suppose that the lightning flash has destroyed fibrin ferment and its allies. Among the latter must be counted the alexins. Consequently, decay-producing bacteria might penetrate the tissues with more than ordinary rapidity. As this somewhat vague possibility was among the suggestions that originally led me to seek for a bacteria-killing proteid among the allies of fibrin ferment, I have recently thought it worth while to subject the matter to an experimental investigation. As lightning flashes are not included in the armamentarium of the laboratory, I had to be content with sparks from a six-plate Wimshurst electrical machine, for the use of which I owe my best thanks to Professor J. J. Thomson.

I may here point out that such sparks, though containing electricity of very high potential, contain it in such small quantity that the thermal, electrolytic, or other physical changes they are capable of producing are extremely small, and could only be measured, if at all, by extremely delicate physical methods. Consequently their action on alexin solutions—which I shall describe below—cannot be explained by a reference to the more ordinary effects of an electric current. First, with regard to the action of such sparks on the bactericidal power of serum. In the case of rabbit's serum it must be trivial or non-existent, for it could not be demonstrated by the following method employed by me for estimating the bactericidal power of serum:

A rabbit's throat is cut and the blood is allowed to flow directly into a sterilised centrifuge tube. As soon as clotting has occurred the clot is loosened from the walls of the tube by means of a sterilised glass rod. The tube is loosely plugged with cotton-wool and placed in a centrifugal machine for some minutes. By this means the serum is expressed from the clot in greater quantity, and more rapidly than would be the case if the serum were left to itself. The serum is then decanted by means of a 1 c.c. pipette into a number of small test tubes that have previously been sterilised and plugged. Exactly 1 c.c. of serum is placed in each tube. The tubes are then inoculated with varying quantities of a vibrio Metschnikovi culture.² A 24-hour-old bouillon growth should be em-

¹ On a Bacteria-killing Globulin, *Proceedings of the Royal Society*, vol. 48, p. 93, May 21st, 1890. On the Conflict between the Organism and the Microbe, *BRITISH MEDICAL JOURNAL*, July 12th, 1890; and On Immunity, read before the Congress of Hygiene and Demography, London, August, 1891.

² The vibrio Metschnikovi is preferable for this method, and the anthrax microbe for the plate culture method of estimating the bactericidal power of alexin solutions, for reasons mentioned later on in this paper.

ployed. It must first be well shaken. Then 0.01 to 0.02 c.c. of it is removed by means of a graduated pipette, and mixed with 10 c.c. of a sterilised normal salt solution. The graduated pipette is again sterilised by immersion in boiling water, and used to inoculate the serum tubes with gradually-increasing quantities of the diluted culture. Perhaps the first test tube of the series receives 0.005 c.c. of the diluted culture; the next 0.01 c.c., and so on till the last tube receives 0.1 c.c. of the original undiluted culture. This last tube will certainly produce a growth after twenty-four hours, if the serum of ordinary rabbits is employed. A varying number of the other tubes will remain sterile. Perhaps the tube containing 0.03 c.c. of the diluted culture will have produced a faint indication of a growth. Tubes inoculated with larger quantities will be found to show typical growths after twenty-four hours. Those, on the other hand, inoculated with smaller quantities appear to be sterile. In this way a rough estimation can be obtained of the quantity of the culture that 1 c.c. of the serum in question is capable of destroying. This method is only applicable to liquids showing a well-marked bactericidal power, but is easier to carry out than the plate culture method.

When tested in this way rabbit's serum, through which ten to fifteen loud sparks from a Wimshurst machine had been passed, showed just as great a bactericidal power on anthrax and on vibrio Metschnikovi as normal serum.

In the case of rat's serum I have, however, obtained evidence that sparks of high potential can affect its bactericidal power to a slight degree, as shown by the following experiment:

Three rats were killed by decapitation, and their blood collected in the same vessel, which was kept in a cool place till the next day to permit the separation of the serum. Part of this serum was mixed with virulent anthrax spores from a fresh agar culture, and immediately injected in quantities varying from 0.02 to 0.08 c.c. Of these mice four survived. A control mouse inoculated with anthrax alone died within eighteen hours. Another portion (less than 1 c.c.) of the same serum was placed in a small sterilised U-tube, where it formed a column about 1 centimetre long. Two stout platinum wires were taken. One end of each was coiled up into a little ball. One was passed down each arm of the U-tube till the coiled end was just immersed in the serum. The wires were held in position by plugs of sterilised cotton wool. The wires projected about 2 inches out of the ends of the tube, and these free ends were furnished with little brass balls about half an inch in diameter. One ball was placed in contact with one pole of the Wimshurst machine. The other ball was kept at 2 to 4 inches distance from the other pole, and six loud sparks were allowed to pass. This "sparked" serum was then mixed with spores, and injected into five mice exactly as in the first part of the experiment. Of these only one survived, showing that the power of the rat's serum to prevent the development of anthrax in mice was to a great extent removed by the passage of a few sparks of high potential. The serum used in this experiment had a faint reddish colour, and showed the spectrum of oxyhemoglobin both before and after the passage of the sparks. The sparks also were without effect on its chemiotactic action on the leucocytes of the mouse.

In another similar experiment of four mice inoculated with normal rat's serum and anthrax spores only two survived, while of four inoculated with "sparked" serum everyone succumbed.

In another experiment, however, in which the serum in the U-tube was in a longer column, and in which shorter sparks were employed, no result was obtained; all the mice, whether treated with "sparked" and normal serum, surviving, only the control mouse dying in the usual time.

With pure alexin solutions, on the other hand, very clear proof can be obtained of the action of electric sparks on their bacteria-killing activity. The bactericidal action of such solutions is completely removed by the passage of a few electric sparks, as shown by the following experiments:—

1. October 20th. The spleen of a dog immediately after the death of the animal was cut out, triturated in a mortar, and a quantity of alcohol was added. The next day the alcohol was filtered off, and the residue was extracted with 20 c.c. of a one-tenth saturated sodium sulphate solution till October 22nd. The solution was then filtered, and a large quantity of alcohol was added. On November 3rd the precipitate that had been formed by the alcohol was filtered off and dried. On November 4th it was pounded up and extracted with 10 c.c. of 0.75 per cent. NaCl solution that had been made faintly alkaline with sodium phosphate, and put in a shaking machine for six hours; then another 10 c.c. of 0.75 per cent. NaCl solution was added, and the liquid was kept in ice till November 6th. On this day, after filtering, it was dialysed for one hour and a half against two litres of sterilised 0.75 per cent. salt solution at a temperature of 42° to 43° C. All these manipulations were carried out under strict antiseptic precautions. After the dialysis the liquid was divided into two equal parts. Of these one was placed in a small sterilised U-tube. A series of sparks from the Wimshurst machine of the length of from 8 to 12 centimetres were passed through the liquid, as in the experiments above described. The alexin solution was then poured into a sterilised test tube, and, with the other portion which served as control, tested for a bactericidal power by the plate culture method. The results were as follows:—

Numbers of Colonies on:			
	Control Plate.	Plate made after ½-hour.	Plate made after 1 hour.
Normal alexin solution	194	11	8
Alexin solution after passage of sparks	120	226	746

Thus the normal alexin solution exhibited a bactericidal power, while a similar solution which had been subjected to

sparks from an electrical machine proved itself an excellent culture medium. Still more striking than the results of counting the colonies were the conditions of the two test tubes on the day after the experiment had been made. The tube containing the normal alexin solution showed no apparent growth. A slight sediment was, however, present which under the microscope showed anthrax bacilli apparently commencing to develop. The tube containing alexin solution through which sparks had been passed showed, on the contrary, a copious and typical growth. Another experiment gave a precisely similar result as follows:—

2. The spleen of a dog was chopped up with 150 c.c. of alcohol. After half an hour the alcohol was filtered off, and the residue was extracted with one-tenth saturated sodium sulphate solution, and placed in a shaking machine during the night. The next morning (October 17th) it was filtered and precipitated with several times its volume of alcohol. On November 12th about half this precipitate was freed from alcohol by filtering and drying. It was pulverised in a mortar, and 15 c.c. of normal salt solution was added at 11.45 A.M. It was kept at a temperature of 37.5° C. till 12, and then placed in a shaking machine till 1.45 P.M. It was then dialysed till 3.30 at a temperature of 44° to 48° C. against sterilised normal salt solution; then it was filtered and divided into two parts: one of these, as in the former experiment, had 8 or 10 sparks passed through it. Both parts were then tested as to their bacteria-killing power, with the following result:—

Numbers of Colonies on:			
	Control Plate.	Plate made after ½-hour.	Plate made after 1 hour.
Normal alexin solution	700	436	168
Alexin solution after passage of sparks	562	742	*

* Colonies so numerous that they could not be counted owing to liquefaction of the gelatine.

On the following day both tubes presented an anthrax growth.

Similar results were obtained with the alexin of the rabbit, as shown in the following experiments.

The spleens of two rabbits were ground up in a mortar with powdered glass, and then left under alcohol for three days. The alcohol was then filtered off and the mass extracted with 10 c.c. of a one-tenth saturated sodium sulphate solution for some hours. After filtering the liquid was mixed with several times its volume of alcohol. The precipitate was filtered off and dissolved in 5 c.c. of 0.75 per cent. sodium chloride solution. It was then dialysed at a temperature of 38° C. for two hours, and again filtered. The solution was then divided into two equal parts, of which one received six sparks, the other served as control. In this experiment vibrio Metschnikovi and not anthrax was employed. Of a twenty-four-hour-old bouillon culture, 0.005 c.c. was taken, and well mixed with 10 c.c. of a 0.75 per cent. NaCl solution. Of this diluted culture about 0.25 c.c. was used to inoculate each of the tubes of alexin solution, and of the latter 0.1 c.c. was taken for inoculating each of the plates. Agar at 42° was used instead of gelatine, and the plates were kept at 33°, till the next day, when the colonies were counted with the following results:—

Numbers of Colonies on:			
	Control Plate.	Plate made after ½-hour.	Plate made after 1 hour.
Ordinary alexin solution	70	11	4
Sparked alexin solution	84	77	107

The progressive diminution in the numbers of colonies from the ordinary alexin solution shows that the greater part of the microbes contained in it were killed within an hour. On the other hand the alexin solution through which sparks had been passed had not the slightest power of killing the microbes, or even of delaying their development as shown by the progressive increase in the number of colonies on the different plates.

Another experiment gave a similar result.

These experiments are, I think, sufficient to prove that a few electric sparks of high potential are capable of robbing a pure alexin solution of its power of killing bacteria. Whether solutions of ordinary digestive ferments lose their powers when subjected to a similar treatment would, I think, be an interesting matter for research.

This action of electric sparks on alexin solutions has a certain theoretical interest for the following reason. When it was first shown that blood serum possesses a power of killing bacteria, it was suggested that we were here dealing merely with the effects of a "change of medium." Haffkine³ showed that the bacillus of typhoid fever is killed by an abrupt removal from bouillon to aqueous humour. Further, that it can be habituated to living in the latter medium, and in this condition it is killed by removal into ordinary bouillon. The latter now exerts a "bactericidal power" on the microbe in question. However the "change of medium" criticism may apply to the bactericidal action of blood serum,

³ Recherches sur l'Adaptation au Milieu chez les Infusoires et les Bactéries, Annales de l'Institut Pasteur, June, 1890, p. 363.

it applies with still greater effect to the bactericidal power of alexin solutions which may contain but a trace of nutrient substances in solution, and which may exhibit a very limited power of destroying bacteria.

If I urge that anthrax bacilli are no longer killed by being placed in alexin solution that has been previously heated, this scarcely disposes of the difficulty. No doubt the passage from bouillon to boiled alexin solution is a change of medium, but the fact that they can survive one change of medium does not prove that they are immune against other changes of medium, of which the passage from bouillon to unboiled alexin solution may be quoted as an example. Besides, in the process of heating an alexin solution various physical changes are brought about—coagulations, removal of gases, etc.—which may very well cause it to approximate in nature to the bouillon in which the bacilli have been living, and so prevent the usual effect of a sudden change of medium. Again, if I urge that keeping an alexin solution for some days at a temperature of 37.5° C. causes it to lose its bactericidal power, it may be retorted that various physical alterations may have occurred in consequence of which it no longer presents a dangerous change of medium to bouillon-grown bacilli. Kitasato has shown that if bouillon is kept for some days before use it almost loses its power of supporting the growth of the tetanus microbe. Obviously this alteration is due to some physical change. No one would suggest that it was due to the development of a specific bactericidal power. Conversely, why assume the removal of a specific bactericidal power to explain how an alexin solution becomes by keeping fit to support the growth of microbes?

In reality such objections have less force than may at first sight appear. What is really implied when we say that a microbe has been killed by a change of medium can be illustrated by a couple of examples. If we take some tetanus microbes from an animal dead of the disease, and attempt unsuccessfully to cultivate them in stale bouillon, we may say that the microbes have been killed owing to the change of medium. Supposing we take some anthrax bacilli from a fresh bouillon culture and place them in bouillon that has been saturated with chloroform the microbes equally perish, and their death is also obviously due to the change of medium. There is, however, a difference in the two cases. In the first case the microbes have been subjected to a completely new set of biological conditions. They have been unable to adapt themselves to the circumstances of the environment and consequently have perished. To ascribe their death to the change of medium is both justifiable and convenient. In the latter case the microbes have not been subjected to a completely new set of conditions. Only one condition, namely, the presence of the chloroform, is hostile to the microbes. In this case, to ascribe the death of the bacilli to the change of medium is correct but inconvenient. To ascribe their death to the presence of a bacterial poison is the simpler mode of expressing the facts of the case. That the latter mode of expression more conveniently describes the cause of death of anthrax bacilli in alexin solutions is well shown by the effect on the latter of electric sparks of high potential, as above described.

In these experiments the property on which the bactericidal action of alexin solution depends is removed by a means which is not known to produce any other physical change in the medium. Anthrax bacilli from a bouillon culture, when placed in such a "sparked alexin" solution, are subjected to a "change of medium" apparently as great as any incurred by them in Haffkine's experiments, but yet the plate-culture method shows that no diminution in the number of living bacilli occurs. It is obviously still open to anyone to say that anthrax bacilli, when placed in an alexin solution, are killed owing to a change of medium. The same mode of expression would lead one to say that a patient killed by an overdose of arsenic had succumbed to a "change of diet." But in both cases to ascribe the result to a specific poisonous action is the more convenient nomenclature. Nor is the case in any way changed by urging that, because anthrax bacilli can be gradually acclimatised to living in an alexin solution, therefore their death on their first introduction into it was merely due to the change of medium. A patient may be gradually so accustomed to arsenic that it becomes an integral and even necessary part of his diet. Yet no one would say because of this that arsenic is not a poison.

I do not at all wish to deny that bacteria may perish owing to the effect of a "change of medium" in the absence of a specific bactericidal substance. I think that Haffkine's experiments with the typhoid bacillus form a striking example of this. The typhoid bacillus, when mixed with the aqueous humour, is killed. That the result is not due to the presence of any specific bactericidal action is shown by the fact that, when tested with other microbes, such as the bacillus of anthrax, the vibrio Metschnikovi, the bacillus of quarter evil, or the bacillus of swine erysipelas, aqueous humour has been found by various authors to be an excellent culture medium, although serum from the same animal may exert a well-marked bactericidal action on the microbes mentioned.

The anthrax bacillus can, as has been shown by Buchner, withstand such a sudden "change of medium" as removal from a mouse's spleen to a solution containing 40 per cent. of sugar and 1 per cent. of peptone. I have often noticed that if 0.1 c.c. of a fresh bouillon culture of anthrax is placed in 10 c.c. of 0.75 per cent. NaCl solution a copious growth is apparent after twenty-four hours. These and many other facts point out that the anthrax bacillus is one well capable of resisting the action of a sudden change of medium, and this is why I have almost exclusively worked with this microbe in investigating the specific bactericidal power possessed by alexin solutions.

Lastly, I may mention that Christmas⁴ has suggested that the bactericidal power of blood serum may be due to the presence of carbonic acid gas. This idea scarcely agrees with von Fódor's observation⁵ that serum from arterial blood has a greater bactericidal power than that obtained from venous blood, and, further, that the bactericidal power of serum is diminished by exposure to an atmosphere of CO₂. In any case there is no reason for thinking that the purified alexin solutions with which I have worked contain any appreciable quantity of this gas.

REMARKS ON A CASE OF CHOLERA AT LIVERPOOL.

By THOMAS WHITESIDE HIME, B.A., M.D.,
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INHUMAN indeed would be the heart which is not stirred by deepest sympathy for the inhabitants of those localities which have recently been attacked by this direful scourge. One knows but little of the distant, dumb sufferings of the great Russian empire (so different from the hysterical excitement shown in another country at the mere idea of approaching danger) beyond the fact that for months past the vitality of its vast population is being sapped by cholera. Nearer to ourselves is Hamburg, which is like a city in which a terrific conflagration had suddenly burst forth, whereof the sparks are being carried broadcast, causing imminent danger far and wide.

Wehe, wenn sie losgelassen,
Wachsend ohne Widerstand,
Durch die volkbelebten Gassen
Wälzt den ungeheuren Brand!

There is some tendency in the public press of this country to a pharisaical exultation that we are not as other men; that we, having swept and garnished, drained and disinfected in good time, have definitely escaped the cholera as a natural reward for our prescience and forethought.

This frame of mind is one of the dangers of the situation, and is only too well calculated to breed a sense of false security and negligence. The indications are happily that just now the combination of conditions which produce susceptibility to epidemic cholera does not exist among us. But how long will this last? No one can tell. But every experienced person does know that one of the most potent and necessary factors for the spread of epidemic cholera is absolutely under our control; we ourselves produce the filth which in the air, in the soil, in our drinking water, in our houses, and about our persons, not only induces low condi-

⁴ Etude sur les substances microbicides du sérum et des organes d'animaux à sang chaud, *Annales de l'Institut Pasteur*, vol. v, August, 1891, p. 487.
⁵ Neuere Untersuchungen über die bacterientödtende Wirkung des Blutes und über Immunisation, *Centralblatt für Bakteriologie*, vol. vii, pp. 753 et seq.