EXPERIMENTS ON THE PREVENTIVE INOCULATION OF RATTLESNAKE VENOM.

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THE following work was undertaken with the hope that it might form a worthy contribution to the theory of Prophylaxis, and the results obtained during the first stage of its progress are put forward at this time because of the impression that, perhaps, at least their practical significance may induce investigators more fortunately situated for the performance of such experiments to take up the same line of observation. I have assumed an analogy between the venom of the poisonous serpent and the ptomaines produced under the influence of bacterial organisms. Both are the outcome of the activity of living protoplasm although chemically widely distinct, the ptomaines belonging to the group of alkaloids, while the active principles of the venom, according to Mitchell and Reichert[1] and to Wolfenden[2] are of proteid nature.

If immunity from the fatal effects of snake-bite can be secured in an animal by means of repeated inoculation with doses of the poison too small to produce ill effects, we may suspect that the same sort of resistance against germ-disease might follow the inoculation of the appropriate ptomaine, provided that it is through the products of their metabolism that bacteria produce their fatal effects. It is not necessary at this time to consider the bearing of the literature on the subject in question, for there can be drawn from it few, if any, unassailable conclusions.

It is a matter of common experience that with the repeated exhibition of various kinds of poisons in therapeutic doses, more and more of the substance must be employed to produce its physiological action, and, finally, ordinarily fatal doses may be given with impunity. And yet there is reason to believe that this resistance may result from either of two opposite conditions impressed upon the living parts of the body, a pathological or a physiological.

In the first case the sum total energy of the protoplasm is diminished; its irritability is lowered as well as its efficiency as a machine. In the second case the total energy of the protoplasm is not diminished

[1] "Researches upon the Venoms of Poisonous Serpents." *Smithsonian Contrib. to Knowledge*, 674, 1886.

[2] This *Journal*, Vol. VII. p. 327.

but, perhaps, is even increased as the effect of the inexplicable tendency of living matter to adjust itself to its environment; such a physiological resistance is shown by the secretory cell which does not digest itself, and by the unicellular animals which dissolve ingested matter but spare their own substance. Whatever want of value there may be in these considerations, definite facts upon the subject discussed in this paper have a worth of their own.

The venom used in these experiments was obtained from three specimens of the Massasanga, *Crotalophorus tergeminus*, one of the smallest of the rattlesnakes. The average length of the reptiles was about two feet. In order to obtain the poison the snake was allowed to bite the side of a porcelain dish while being held round the neck by a loop fastened to the end of a stick. The number and variety of the experiments performed were narrowly limited by the small amount of venom obtained. After the first extraction of poison, only one snake gave an additional amount; nor did artificial feeding with chopped meat induce a further secretion.

It was sought to artificially excite the secretion by stimulating with an induction current the exposed poison gland and its nerves. The results, on the whole, were negative, though in one instance stimulation of the gland was followed by a slight flow of venom into the capillary tube inserted in the poison duct, and each application of the electrodes caused a greatly accelerated blood-flow from the cut muscles which had covered the gland. The total amount of venom obtained was estimated to be about six small drops, though as part of it dried after extraction an accurate measure was impossible. The six drops of venom were dissolved in about 88 drops of glycerine, as recommended by Weir-Mitchell[1], so that each drop of the glycerine-venom contained ·068 drop of the pure venom. The venom was clear and greenish-yellow in colour and decidedly acid in reaction. In preparing for an inoculation, the requisite number of drops of glycerine-venom were allowed to fall into a porcelain capsule and diluted with distilled water to such an extent that each animal should receive 15 minims of the mixture.

Mitchell and others have found that pigeons are peculiarly sensitive to the influence of rattlesnake poison, and these birds were therefore uniformly used in the experiments. The inoculations were made under the skin of the back by means of a hypodermic syringe.

In no case did death follow injection so rapidly as described in some

[1] "Researches on the Venom of the Rattlesnake." *Smithsonian Contrib. to Knowledge*, 1861.

of the experiments of other workers on this subject; this result may have been due either to the high dilution of my poison or to its intrinsic composition. There appeared to be two well-marked periods within which death followed after inoculation with the venom; the shorter time was about 3 hours, and the longer 15 to 20 hours.

The first symptom of the action of the poison was invariably a weakness in the legs, inducing the pigeon to sit down or to move with a tottering gait when forced to rise. Complete paralysis of the legs follows a larger dose of the venom. The general health and appetite of the bird may remain apparently unaffected while the legs are nearly completely paralyzed. Another symptom of the poisoning is the apparently excessive lachrymal secretion. When a fatal dose of poison is given, the paralysis extends from the legs to the wings; the head rests upon the floor; the mouth is open and the respiration gasping, and, after a longer or shorter period of clonic convulsions, the pigeon dies.

Mitchell draws attention to the marked effect which the inoculation of rattlesnake venom has in rendering the blood incoagulable, in producing extensive extravasation from the blood-vessels and in hastening putrefactive decomposition with the formation of very offensive odours. None of these results could be verified, perhaps on account of the dilute condition of the poison with which this work was done.

Previous observers seem to agree that snake venom may be kept indefinitely without diminution of its virulence; but, in the absence of quantitative experiments to the contrary, I think my own results are valid as showing that rattlesnake venom preserved in glycerine undergoes a gradual deterioration in its power. On consulting the tabular statement of the experiments on prophylaxis, it will be seen that on Nov. 7 the fatal dose of glycerine-venom was $\frac{2}{5}$ drop; on Dec. 2 it had risen to $\frac{2}{3}$ drop; and on May 19 nearly 1 drop was injected into a pigeon without producing death. A yellow colour, such as occasioned by fat, marked the skin for a considerable area round the needle punctures. In some of the experiments the pigeons inoculated with the same amounts of venom were divided in two lots, those of one being preserved in a room kept fairly constantly heated to about 60° F., while the others were caged in an unheated room in which the temperature frequently descended to 0° F. The poison seemed to affect more seriously those pigeons preserved in the cold room.

There appeared to be a maximal dose of venom which produced death as quickly as any greater amount, illustrated by the following experiments:

EXPERIMENT 1.

Nov. 4. At 2.12 p.m. injected 3–4 drops glycerine-venom in water under skin of a pigeon. At 4.10 found the bird lying on the floor of its cage, legs paralyzed. At 4.15 the bird remains on its back when so placed; heart rather irregular and slow; body cooling. Lachrymal secretion. Slight convulsions. Died at 4.25, in 2 hours 13 minutes. Blood clots fairly well.

EXPERIMENT 2.

Nov. 4. At 3.25 p.m. injected about 12 drops glycerine-venom. At 4.10 bird still unaffected. At 4.50 fell to the floor, beginning paralysis in legs. Can move the wings strongly and fly when thrown in the air; will not lie on its back. At 5 p.m. convulsions, pigeon dying fast. Still alive at 5.25 but found dead next morning.

Thinking that the fatal effects of the poison might be due to some sort of ferment action of the venom, half a dozen drops of the blood from the pigeon of experiment 1 were injected into each of two fresh birds. The result was negative.

A number of inoculations were made upon various pigeons to determine the minimal fatal dose of the glycerine-venom mixture, and then it was sought to discover whether repeated inoculations with subminimal but continually increasing doses of the poison would produce immunity against the fatal effects of unlimited amounts of the virus, and whether such immunity might be merely transient or persistent in its character. The action of the poison in the early stages of a series of observations was to some extent cumulative; for a dose too small to alone produce death could become fatal if given to a pigeon which had not yet recovered from a previous inoculation.

The results of the prophylactic inoculations are given below in a tabular form. The experiments were carried out on seven pigeons, each of which is designated by a separate Roman numeral and its history can be followed by glancing at the left-hand column of the table. The expression I—VI means that 6 pigeons from I to VI were inoculated at the same time with like doses; IV—VI means that birds IV, V and VI were inoculated, etc. The date of the injection is noted in the second column from the left, and the amount of glycerine-venom, in drops, given to each pigeon, diluted for each bird with 15 minims water, is recorded in the third column.

From time to time control experiments were made on fresh pigeons. The tabular results are noted on the right-hand side of the table. The column on the left indicates the number of pigeons employed, and the next column to the right the dose given to each bird.

Experiments on Prophylaxis				Control Experiments		
Title of pigeon	Date of inoculation	Dose of glycerine-venom for each in drops	Remarks	Number of pigeons	Dose of glycerine-venom given each in drops	Remarks
	1886 *Nov.* 6					
I	10.30 a.m.	$\frac{1}{5}$	Result negative.	4	$\frac{1}{5}$	Results negative. In some the skin becomes greenish under the place of puncture.
	Nov. 7					
I	10 a.m.	$\frac{2}{5}$	Next morning bird found lying gasping, legs paralyzed, eyes watering. Occasional clonic convulsions. Pigeon fed by hand, not recovered till Nov. 12.	4	$\frac{2}{5}$	All found dead on morning of Nov. 8.
	Nov. 9					
II—VII	9.30 a.m.	$\frac{1}{6}$	No effect in 24 hours.			
	Nov. 10					
II—VII	10 a.m.	less than $\frac{1}{3}$	In 24 hours all six affected but only two seriously. Pigeon VII found dead 3.30 p.m. Nov. 11.			
	Nov. 12					
I—VI	9 a.m.	$\frac{1}{6}$	Pigeons II—VI still weak from previous inoculation. Six hours after inoculation VI is paralyzed in legs and two others are ill. All but VI are well Nov. 13.			
	Nov. 14					
I—IV	10.15 a.m.	less than $\frac{1}{4}$	One, IV, is affected in 24 hours.			
	Nov. 15					
I—III	9.30 a.m.	$\frac{1}{3}$	In 24 hours all are partially paralyzed in legs. Slow recovery.			
	Nov. 15					
V and VI	9.30 a.m.	$\frac{1}{4}$	Pigeons not affected.			

Table (*continued*).

Experiments on Prophylaxis				Control Experiments		
Title of pigeon	Date of inoculation	Dose of glycerine-venom for each in drops	Remarks	Number of pigeons	Dose of glycerine-venom given each in drops	Remarks
IV—VI	*Nov.* 16 10 a.m.	$\frac{1}{3}$	In 24 hours IV—VI are but slightly affected. I is still weak in legs. On Nov. 18 all completely recovered.			
I—VI	*Nov.* 19 9.30 a.m.	less than $\frac{1}{2}$	In 5 hours one bird, VI, found dead, and one other affected. In 24 hours only IV and V are affected. On Nov. 22 all, I—V, are well.			
I—V	*Nov.* 22 9.30 a.m.	$\frac{1}{2}$	No effect.			
I—V	*Nov.* 23 4 p.m.	more than $\frac{1}{2}$	No effect.			
I—V	*Nov.* 24 10.30	$\frac{3}{5}$	In 24 hours only V slightly affected.			
I—V	*Nov.* 26 10 a.m.	$\frac{4}{5}$	No effect.			
I—V	*Nov.* 27 9.30 a.m.	1	No effect.	2	less than $\frac{1}{2}$	In 24 hours one bird (1) has legs paralyzed; not recovered till Dec. 2. The other bird (2) is unaffected.
I	*Nov.* 29 10.30 a.m.	2	No effect.	1	1	Pigeon (2) of previous exp. Found dead in 6 hours.
II—V	*Dec.* 2 11.30 a.m.	2	No effect.	$\frac{2}{3}$	3	Including bird (1) recovered from previous exp. This bird found dead in 6 hours, the other two dead next morning.

Table (*continued*).

Experiments on Prophylaxis.				Control Experiments		
Title of pigeon	Date of inoculation	Dose of glycerine-venom for each in drops	Remarks	Number of pigeons	Dose of glycerine-venom given each in drops	Remarks
I	*Dec.* 3 9.30 a.m. 1887	less than $4\frac{1}{2}$	No effect.			
I—V	*Jan.* 6 11.30 a.m.	3	Next morning V found dead. I is somewhat weak.	1	1	This bird had been kept 2 months in cage without inoculation. Found dead next morning.
I	*April* 28 10.30 a.m.	less than 3	Slightly weak in 24 hours.	1	less than 1	Paralyzed on same afternoon. Found dead next morning.
III	*April* 29 3.15 p.m.	2	Legs paralyzed in 15 minutes. Found dead next morning.	1	less than 1	Found dead next morning.
IV	*April* 29 3.15 p.m.	3	Found dead next morning.			
II	*May* 6 9.45 a.m.	more than 1	Slightly weak in 24 hours.			
II	*May* 16 9.45 a.m.	2	No effect.			
II	*May* 18 11 a.m.	3	No effect.			
II	*May* 19 11.30 a.m.	4	No effect.	1	less than 1	Young pigeon; partially paralyzed in 24 hours, but finally recovers.

A review of the foregoing table of experiments shows that repeated inoculation of pigeons with sub-lethal doses of rattlesnake venom produces a continually increasing resistance towards the injurious effects

of the poison without apparent influence on the general health of the animals. On Dec. 3, when the fatal dose of glycerine-venom is less than $\frac{2}{3}$ drop, $4\frac{1}{2}$ drops, or about 7 times the fatal dose, were injected without the least effect into pigeon I. The efficiency of resistance against the venom gradually fails in absence of fresh inoculation, as is witnessed in the case of pigeon I which is perceptibly weakened by the inoculation on Jan. 6 of 3 drops of glycerine-venom, a little more than one month after $4\frac{1}{2}$ drops had been injected without effect. Also on April 29, nearly five months after the next preceding inoculation of 2 drops of the poison, III and IV were killed by inoculation, the one with 3 and the other with 2 drops of the poison. That the prophylactic effect of the repeated inoculations is persistent over the interval of five months is shown by the example of pigeon II, which on May 6 is uninjured by a fatal dose of the poison, and thereafter rapidly recovers its powers of resistance against the ill effects of inoculation with doses of venom much in excess of the fatal amount.