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# THE PRODUCTION OF IMMUNITY WITH THE CHEMICAL SUBSTANCES FORMED DURING THE GROWTH OF THE BACILLUS OF HOG-CHOLERA.

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As a continuation of the preliminary paper on the ptomaïnes from the hog-cholera germ, presented by us to the Chemical Section of the American Association for the Advancement of Science, in August, and published in THE MEDICAL NEWS, September 6, 1890, we present now a somewhat detailed account of the successful experiments in the production of immunity in guinea-pigs which have been made up to date. The work from this standpoint again is of course a practical continuation of the experiments of Drs. Salmon and Smith, made upon pigeons in 1887, in which sterilized culture-media were used for preventive inoculation. We refer further to the bulletin of the Bureau on "Hog-cholera," published in 1889, in which are recorded a number of experiments upon hogs, sterilized culture-media being used for the purpose of producing immunity.

This work of Drs. Salmon and Smith was the pioneer work in preventive inoculation with other than some form of the germ of the disease itself,

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and the work now recorded was of course under the advice and direction of Dr. Salmon as head of the Bureau of Animal Industry. Without the careful bacteriological study of hog-cholera which has been made by the Bureau of Animal Industry, our work would have been impossible. For our laboratory experiments guinea-pigs were used, as being convenient to handle and susceptible to hog-cholera. They have proved very satisfactory. The material used for inoculation was prepared in the chemical laboratory by modificatioms of methods already described, and by other methods which will be explained in more detail at some future date. The testing of the materials used, to determine that they were free from germs, and the greater part of the preventive inoculations, were made by Dr. Moore, with such quantities of substance and at such times as we thought best. The autopsies were also made by Dr. Moore, and the work thereby greatly facilitated.

As to the name which should be given to the ptomaïnes and albumins from the hog-cholera culture-liquids, until their chemical constitution is more thoroughly studied, it would seem best, as there are several distinct swine-diseases, to call the ptomaïnes from the hog-cholera germs, as a class, *Sucholotoxins*, and the ptomaïne which appears to be the principal factor *Sucholotoxin* (from the Greek Σῦς, a hog, Χολέρα, cholera, from Χολή, bile, and *toxus*, poison). *Sucholo-albumin* would seem to be sufficiently distinctive for the albumin of these culture-liquids. As Hankin [1] shows, the name *toxalbumin* is hardly

[1] British Medical Journal, 1889, p. 810.

the correct one to apply to these substances. We shall, therefore, refer to the ptomaïnes and albumin by the names given above.

The first of our experiments that we will record were made with sucholotoxin.

EXPERIMENTS I.—Two guinea-pigs, each weighing about three-fourths of a pound, were treated with a solution of about 0.05 gramme of sucholotoxin each. The solution was introduced under the skin of the inner side of the left thigh. Immediately after the operation the animal appeared uncomfortable, but was not made ill. For a few days there was a rise in temperature and also a slight swelling at the point of inoculation, which, however, disappeared in about five days, and the animal was then well.

Two more guinea-pigs were now selected as checks, approximately of the same size and weight as those which had been treated, and the four animals were then inoculated with 0.1 c.c. of hog-cholera virus each (0.1 c.c. beef-infusion and peptone culture one day old, plus 0.2 of sterile, normal salt solution). This is the dose which previous experiments made in the Bureau had shown to be the proper quantity to kill a guinea-pig in from eight to ten days. The inoculations with the virus were also made subcutaneously in the thigh. The checks died in eight and nine days. Post-mortem examination showed a large swelling at the point of inoculation, infiltration of a purulent, grayish substance into the connective tissue, and necrosis of the superficial layer of the muscles of the thigh. Enlargement and reddening of inguinal glands. Peyer's patches enlarged and pigmented; liver pale and covered with

a number of necrotic foci; spleen very much enlarged, dark-colored, and friable. Cover-glass preparations from the spleen and liver showed hog-cholera germs. This was the characteristic appearance of all the check guinea-pigs upon post-mortem examination, and it will not be necessary to repeat these details.

Of the animals which had been first treated with the substance mentioned, and afterward inoculated, one died two days after the last check. Autopsy revealed the following: At the point of inoculation in the left thigh the subcutaneous tissue was infiltrated with a grayish-white substance, and the superficial layer of muscles over the inner side of thigh, and 4 square centimetres of the abdominal wall were necrosed. Liver pale. Spleen much enlarged, dark-colored, and friable. Cover-glass preparations from the spleen showed a large number of hog-cholera germs. Both ventricular walls of the heart were light-grayish and very brittle (necrosed). The other guinea-pig of this set was quite ill for ten days, with a large swelling at the point of inoculation. This finally opened and healed and the animal was quite well within three weeks after the inoculation, and has continued so to date—five months.

Experiments II.—The next series of experiments were made with sucholo-albumin from beef-infusion and peptone culture-media.

Two guinea-pigs were again selected and treated with about 0.008 gramme each of sucholo-albumin. There was a slight rise of temperature in the animals and the formation of a small, hard lump at the point of injection. This disappeared by the eighth day

and the animals were quite well. Two more guinea-pigs were now taken as checks, and all four animals were inoculated with 0.10 c.c. of hog-cholera culture. The checks died within seven days. The post-mortem appearances were practically the same as those noted in the first series. The two guinea-pigs which had been treated with the sucholo-albumin died *ten* days after the checks. This indicates considerable resistance to the disease. Autopsy showed, at the point of injection with the albumin, the subcutaneous tissue thick and reddened. The animals were considerably emaciated. At the point of inoculation a cyst the size of a walnut, and composed of a grayish, purulent substance, was also found. The muscular wall surrounding this was sprinkled with punctiform hæmorrhages. Peyer's patches swollen and pigmented; mucous membrane of small intestine covered with a dry, yellowish, firm layer of mucus; stomach contained a considerable quantity of liquid; liver pale, and showed fatty degeneration; spleen slightly enlarged and dark. Cover-glass preparations showed no germs, but a culture made from the spleen showed hog-cholera germs. Beneath the peritoneum in the region of the spinal column, and in the mesentery was a considerable number of small grayish tubercles. Several other experiments were made by treating guinea-pigs with the albumin in varying quantities, all showing resistance, and subsequently immunity.

EXPERIMENTS III.—Three guinea-pigs were treated with sucholo-albumin, 0.1 gramme being given to each, subcutaneously in the thigh. The albumin for two of the animals was derived from cultures con-

taining blood-serum, the albumose given to the third was from ordinary beef-infusion peptone culture. Ugly ulcers formed at the point of inoculation, which healed, however, in from ten to fourteen days, and the animals with the exception of a slight rise of temperature were well.

Two checks were again selected and the five animals were inoculated with 0.1 c.c. hog-cholera virus. The checks died respectively in eight and ten days from hog-cholera. The animals which had received the preventive treatment were slightly ill for a few days with swelling at the point of inoculation, which finally opened and then healed nicely, and within a week the guinea-pigs were well.

Three weeks after the inoculation, one of these animals was chloroformed and examined post mortem. Not the slightest scar could be discovered, all the organs appeared perfectly normal, and no germs were found.

EXPERIMENTS IV.—Four guinea-pigs were treated, two with a mixture of sucholotoxins, two with sucholotoxin and albumin. The injections were made as before, subcutaneously in the thighs, and at intervals extending over a period of four weeks. The sore caused by each injection was allowed to heal before the next one was made. After the animals had recovered from the last treatment two checks were selected, and the six were each inoculated with $\frac{1}{10}$ c.c. hog-cholera virus. The checks died, one in eight and the other in ten days, the post-mortem examination showing characteristic hog-cholera lesions. The animals having the preventive treatment were ill for about four days, those that received only

the sucholotoxins being more dull than the others. There was also slight swelling at the point of inoculation with the germ, which subsided in ten days, after which the animals were perfectly well, and have remained so—four months.

EXPERIMENTS V.—Six guinea-pigs were inoculated for this experiment, two with solution of the sucholotoxin and four with a solution of the mixed sucholotoxins. The sucholotoxin solution produced only slight local lesions, while the mixed toxins caused ulceration at the point of injection which did not heal for two weeks. The treatment in this case again extended over a period of from three to four weeks. The animals having by this time recovered, the test-experiment with hog-cholera virus was tried. Four of the animals mentioned above were taken—two from each set—and also two checks, and the six were inoculated. The checks died in eight and nine days, the autopsies showing the characteristic conditions of death from hog-cholera. Those that had the preventive treatment were ill and dull for from four to six days after the inoculation. At the point of inoculation there was also some swelling and infiltration, very slight, however, compared with the similar swelling on the checks. In the treated animals the swelling sloughed and healed, and within ten days after the inoculation they were perfectly well. To test the resistance of the animals that had been treated by this method, to ordinary exposure the following experiments were conducted.

EXPERIMENTS VI.—Two guinea-pigs that had received the preventive treatment, two blanks—*i. e.*,

animals that had received no treatment—and two check animals that were inoculated with hog-cholera virus were placed in one large cage. The checks became ill and died in eight or nine days from hog-cholera. During this time the cage was cleaned only three times, so as to give full and free opportunity for contagion. One week after the checks had died one of the blanks became ill, and died within ten days. The autopsy showed hog-cholera lesions. The second blank became ill a few days after the first blank succumbed, and died within thirty days. The animals which had the preventive treatment are now and have been quite well, though continually exposed for five weeks to every opportunity for contagion.

EXPERIMENTS VII.—These experiments are a step in advance of those already recorded, and although not quite so conclusive, indicate that the proper methods have been adopted.

A pure chemical compound prepared synthetically in the laboratory, was used for treating the guinea-pigs. Three animals were taken, and this compound was administered to them by the method already used. There was a slight rise in temperature of the animals and swelling and soreness at the point of injection. After this had healed these animals and two checks were inoculated with $\frac{1}{10}$ c. c. of hog-cholera culture. The checks died in eight and nine days. The animals which had been previously treated became ill, two dying five and six days after the checks. The third entirely recovered.

Post-mortem examination of the two that died showed the following: At the point of inoculation

the skin had sloughed away over an area of 1 sq. cm. The superficial muscular layer was necrosed over an area of about 3 sq. cm. and to a depth of 1 mm., lymphatics in the fold of the knee much enlarged; Peyer's patches enlarged and pigmented; spleen *very slightly enlarged and not discolored;* kidneys reddened; lungs normal. Cover-glass preparation from the spleen showed a few hog-cholera germs. On both sides of the spinal column were several grayish tubercles, from ¼ to 2 mm. in diameter, lying just beneath the peritoneum. This material is being more fully tested, and experiments which promise to be successful are also being made upon hogs. Autopsies made from the animals of Experiments VI., three or four weeks after their recovery, showed that the parts were perfectly normal, not even a scar being left upon the skin, and the immunity produced was therefore *perfect.*

It is important to add that in all the experiments great care was taken that the solutions used were free from germs, cultures always being made. In cases in which the albumin is used this is particularly important. A single precipitation with absolute alcohol does not suffice to destroy the germs, and it is necessary to free the solution from germs by means of a Pasteur filter, or in some other suitable way. Therefore experiments made with material which has not been tested for germs are practically of no value. As to the poisonous character of the ptomaïnes, a single large dose is sufficient to kill a guinea-pig in from one hour to two days. The autopsy of a case of this kind is as follows: Liver, pale and fatty; subcutaneous tissue over abdomen,

necrosed and infiltrated; muscle, soft and friable. Other organs apparently normal.

The experiments here recorded show:

1. That in guinea-pigs *complete immunity* from hog-cholera can be produced by *chemical inoculation.*

2. The sucholotoxins and sucholo-albumin are equally effective in this respect, and a mixture of these two products gives greater immunity than either used by itself. The effect of the albumin in producing immunity from anthrax has already been pointed out by Hankin, his experiments being very successful.

3. The sucholotoxins given in large doses produce death. To produce immunity it is necessary that they should be administered in small quantities at a time and at frequent intervals, the system being in this way accustomed to the poison and enabled to resist it.

Further study in this interesting line of work is in progress.

The tabulated results of the foregoing experiments are appended:

TABULATED RESULTS OF EXPERIMENTS IN PRODUCING IMMUNITY FROM HOG-CHOLERA IN GUINEA-PIGS.

| Number of experiment. | Material used for treatment. | Hog-cholera virus used for each animal. | Number of animals used. | Number of checks. | Number of days between inoculation with virus and death of checks. | Result in treated animals. |
|---|---|---|---|---|---|---|
| I. | Sucholotoxin . . . | 0.10 c.c. | 2 | 2 | 8 and 9 | 1 died in 11 days; 1 recovered. |
| II. | Sucholo albumin . . | do. | 2 | 2 | 7 | Died in 17 days; great resistance. |
| III. | Sucholo-albumin . . | do. | 3 | 2 | 8 and 10 | Recovered; immunity. |
| IV. | 1. Sucholotoxins . . . | do. | 2 | | | Recovered; immunity. |
| | 2. Sucholotoxin and albumin | do. | 2 | 2 | 8 and 10 | |
| V. | 1. Sucholotoxin . . . | do. | 2 | | | |
| | 2. Sucholotoxins . . . | do. | 2 | 2 | 8 and 9 | Recovered; immunity. |
| VI. | Sucholotoxins . . . | do. | 2 | 2 blanks | | Blanks died in 18 and 30 days. |
| | | | | 2 checks | 8 and 9 | Others not affected; immunity. |
| VII. | Pure chemical . . . | do. | 3 | 2 | 8 and 9 | Two died in 13 and 14 days. Third recovered; immunity. |

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