

de Schweinitz (E. A.)

THE PRODUCTION OF IMMUNITY IN GUINEA-
PIGS FROM HOG-CHOLERA BY THE
USE OF BLOOD-SERUM FROM
IMMUNIFIED ANIMALS.

BY

E. A. DE SCHWEINITZ, PH.D.,

BIOCHEMIC LABORATORY, BUREAU OF ANIMAL INDUSTRY, DEPARTMENT OF
AGRICULTURE, WASHINGTON, D. C.



FROM
THE MEDICAL NEWS,

September 24, 1892.

THE PRODUCTION OF IMMUNITY IN GUINEA-PIGS FROM HOG-CHOLERA BY THE USE OF BLOOD-SERUM FROM IMMUNIFIED ANIMALS.¹

BY E. A. DE SCHWEINITZ, PH.D.,
BIOCHEMIC LABORATORY, BUREAU OF ANIMAL INDUSTRY, DEPARTMENT OF
AGRICULTURE, WASHINGTON, D. C.

THE important results obtained by Behring and Kitasato, Tizzoni and Cattani, Klemperer and Klemperer, and others, with diphtheria, tetanus, pneumonia, etc., led me to undertake a series of experiments upon guinea-pigs for the purpose of studying the effect of the blood-serum from animals immune to hog-cholera. As a source for the blood-serum, I used guinea-pigs that had been immunified by means of the albumose extracted from artificial hog-cholera cultures, and then exposed to the disease by inoculation with the virulent germ.² From this latter inoculation the animals had not suffered, and at the time of use had been well for several months. The blood was drawn from the carotid artery into a sterile test-tube, and defibrinated by means of a sterilized glass rod, the overlying serum then being used for injection. Precautions were

¹ Read before the Association of Acting Assistant Surgeons, Rochester, August 15, 1892.

² THE MEDICAL NEWS, October 4, 1890.



taken that the blood did not become contaminated while being drawn, and the absence of germs from the serum was carefully tested.

The experiments were as follows :

Exp. 1. Two guinea-pigs, weighing each about one pound, received three c.c. of this blood-serum by a single injection on the inner side of the thigh. This injection was followed by a slight rise in temperature, but no local lesion at the point of injection, though there was a slight inflammation of the skin for four or five days. After ten days these pigs, together with two checks, were inoculated in the thigh with $\frac{1}{10}$ c. c. of peptonized beef-broth hog-cholera culture, one day old. The checks died in seven days. The autopsy showed characteristic hog-cholera lesions; the hog-cholera bacteria in the spleen were abundant. Both treated animals apparently recovered from the inoculation. Four weeks after the inoculation one of the pigs became paralyzed in the hind legs, and finally died five weeks after the inoculation. The autopsy showed the organs to be apparently normal, except for a few small dots on the surface of the liver. Culture showed an absence of hog-cholera germs.

The second treated pig was found dead three months after the inoculation. The autopsy again showed the organs apparently normal, except for a slight injection of the intestines.

There had in these two cases been then a considerable resistance to the disease from a single injection of the blood-serum. As the germ could not be detected by cultures, and the organs, further, were apparently normal, it is probable that some other cause than hog-cholera caused the death of the animals.

Exp. 2. The experiment was repeated upon exactly the same number of animals, and in the same way, with the result that the pigs treated with the blood-serum recovered entirely, while the checks died. The autopsies of the checks showed characteristic hog-cholera lesions and the germs were abundant in spleen and liver. The experiment repeated again gave the same results—a recovery of the treated animals and the death of the checks.

This work, taken in connection with my earlier results in immunifying guinea-pigs,¹ made it apparent that the blood-serum was a potent factor in the production of immunity.

While in the case of the use of the albumose extracted from the artificial sterilized cultures, or in the use of the simply sterilized cultures, from ten to fifteen c.c., or the equivalent, were required for immunization, here only three c.c. of the serum were necessary.

Treatment of Guinea-pigs Inoculated with Hog-cholera with the Serum from Immunified Animals.—I next tried to cure guinea-pigs with the blood-serum:

Several pigs were taken, and all inoculated with $\frac{1}{10}$ c.c. hog-cholera culture, one day old. Two days afterward two of these animals received an injection of 1.5 c.c. of the blood-serum from an immunified animal. The other animals were reserved as checks. The injections with the same quantity of serum were repeated after two days. The result of the experiment was that while the checks died within ten days, the treated animals resisted for from a week to ten days longer, finally,

¹ THE MEDICAL NEWS, October 4, 1890.

however, succumbing to hog-cholera, as proved by the autopsies.

The experiment was repeated with the same quantity of material, and in the same way, and I succeeded in saving one of the animals, the recovery being entirely satisfactory. All of the checks died. This cure may have been accidental.

At present I am making a more extended series of experiments in this line, beginning the injections with the blood-serum within twelve hours after the infection, and repeating them more frequently. In this way it may be possible to effect a cure in every instance.

Just at this point in my experiments the copy of Pasteur's journal (*Annales de l'Institut Pasteur*, May 25, 1892,) reached my hands, in which a series of experiments upon rabbits is described by Metschnikoff, with the germ which he considers to be identical with that of our hog-cholera. His results were the same as those I had obtained, an easy immunization by means of the blood-serum. Metschnikoff's injections of the blood-serum were all intra-venous and repeated several times. My injections were subcutaneous. The practical results, however, in both cases were the same.

A few tests were made in order to see if the action of the blood-serum was due to its germicidal properties. These experiments showed that the serum *outside of the body* did not exert a germicidal action on the hog-cholera bacillus, and on this point they also agree with Metschnikoff's work.

At the same time that the blood-serum experiments were being carried on I had a number of

guinea-pigs that were being immunified with the albumose extracted from milk-cultures, the products from purely artificial mineral salt and glycerin-cultures, and with the soluble ferments that I have recently obtained from the hog-cholera cultures.

In view of the observations of Roemer¹, Neisser², Canon,³ Botkin,⁴ and Tschistowitsch,⁵ I thought it would be of interest to learn if there was a noticeable increase in the number of white corpuscles in the blood of guinea-pigs inoculated with the germ alone, and in the animals while being treated and after treatment, when inoculated.

The blood was taken daily from the ear of the animal, and the hemocytometer of Thoma-Zeiss employed for estimating the number of corpuscles. Toison's solution served for the staining fluid. Beginning twenty-four hours after each injection or inoculation, the counts were conducted till the animals either died or recovered from the disease. To serve as a check upon the number of white blood-corpuscles, a healthy animal was also used. Comparing the figures thus obtained, allowing for natural daily variation and individual error in counting, we find an increase in the number of white blood-corpuscles, both in the checks and in the treated animals. After the first injection for immunifying the animal, the white blood-corpuscles apparently increased, and then returned to the nor-

¹ Berliner klin. Woch., 1891, No. 36.

² Wiener Med. Presse, 1892, Nos. 3, 4 and 5.

³ Deutsche med. Woch. 1892, No. 10.

⁴ Ibid., 1892, No. 15.

⁵ Berl. klin. Woch., 1891, No. 34.

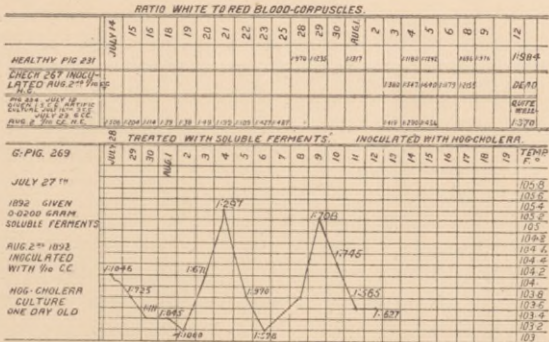
mal as the animal became well. When these same animals were inoculated, the increase of the white corpuscles and return to the normal were equally marked. In the checks, those inoculated with the germs showed a decided increase, and then decrease in the number of white blood corpuscles, till the animal died. These results, though but preliminary as regards the disease of hog-cholera, may be considered as throwing some light on Metschnikoff's theory of phagocytosis.

While writing this article, Kanthack's¹ paper reached my hands. He reports observations upon the leukocytosis produced by the pyocyaneus-cultures, and sterilized cultures of the vibrio Metschnikovi. As his injections were made intra-venously and with fairly large amounts of the material, the increase in the number of leukocytes was more rapid. He observed, upon repeated hourly examination, first a decrease and then an enormous increase in the number of white blood-corpuscles. In my experiments this increase was not quite so marked, nor was the decrease at first noticed, but as the counting of the corpuscles was not begun till twenty-four hours after the injections, it would not have been observed.

In the appended chart is represented the relative number of white and red corpuscles, as found in a healthy guinea-pig, No. 231, in a check, No. 267, and in two treated and then inoculated pigs, Nos. 224 and 269. In the case of No. 269 the corresponding temperatures are also noted. I may

¹ Brit. Med. Journ., June 13, 1892.

take occasion here to acknowledge the help of my assistant, Mr. Emery, in the estimation of the leukocytes.



The results of my work show that there is an easy immunization of guinea-pigs from hog-cholera by means of the blood-serum of immunified animals, and that there is a possible cure of infected animals by a similar treatment. Further, they point to an apparent education of the white blood-corpuses in the blood of guinea-pigs by means of the bacterial products.

The work agrees practically with that of Metschnikoff upon rabbits.

The Medical News.

Established in 1843.

A WEEKLY MEDICAL NEWSPAPER.

Subscription, \$4.00 per Annum.

The American Journal

OF THE

Medical Sciences.

Established in 1820

A MONTHLY MEDICAL MAGAZINE.

Subscription, \$4.00 per Annum.

COMMUTATION RATE, \$7.50 PER ANNUM.

LEA BROTHERS & CO.
PHILADELPHIA.