

## THE PROPHYLACTIC AND THERAPEUTIC PROPERTIES OF THE ANTITOXIN FOR BACILLUS WELCHII.

BY CARROLL G. BULL, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

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In a recent article<sup>1</sup> experiments were given which seemed to establish the following points: (1) under certain defined conditions, a bacteria-free toxic substance can be obtained from cultures of *Bacillus welchii*; (2) this substance possesses the physical properties of an exotoxin and, on animal inoculation, is capable of producing all the essential lesions and effects of infection with the bacilli; (3) animals which have received a number of graded doses of the toxin yield an immune serum which neutralizes *in vitro* all the pathologic effects of the toxin and exhibits power to prevent and control infections with both the spore and vegetative forms of the bacilli. These experimental results appeared to justify the conclusion that *Bacillus welchii* should be classed with *Bacillus diphtheriæ* and *Bacillus tetani* as a toxin-producing organism and that infections with the organism might be successfully combated by means of a specific immune serum. The present paper deals with more extensive and systematic experiments on the preventive and curative powers of the antitoxin.

### *Prophylaxis against Intoxication.*

A series of guinea pigs ranging in weight from 500 to 800 gm. was given a prophylactic dose of antitoxic serum. Each animal received 0.25 cc. of the antitoxin<sup>2</sup> for each 100 gm. of weight, and the susceptibility of the prophylactic series to intravenous injections of the toxin

<sup>1</sup> Bull, C. G., and Pritchett, I. W., *J. Exp. Med.*, 1917, xxvi, 119.

<sup>2</sup> This antitoxin has now been prepared in the horse. The method employed for producing it will be the subject of a later paper, as well as the question of standardization. At present dosage is given in numbers of cubic centimeters of the serum employed.

was compared with that of normal guinea pigs at different intervals after the immune serum had been given. The following protocols give the results:

*Experiment 1. 2 Days after the Prophylactic Dose of Antitoxin.*—Guinea Pig 1, weight 510 gm., had received 1.5 cc. of the antitoxin subcutaneously. 10 cc. of toxin were injected into the jugular vein. No symptoms had developed 6 hours after the injection, but the guinea pig was found dead 20 hours after receiving the toxin. The autopsy showed lesions characteristic of *B. welchii* toxin.<sup>3</sup>

Guinea Pig 2, weight 660 gm., had received 1.6 cc. of antitoxin subcutaneously. 8 cc. of toxin were injected into the jugular vein. The guinea pig developed no symptoms and was discarded in perfect condition 3 weeks later.

Guinea Pig 3, weight 560 gm., normal control. 0.3 cc. of toxin was injected into the jugular vein. This guinea pig was found dead 16 hours later. Characteristic lesions were found at autopsy.

*Experiment 2. 5 Days after the Prophylactic Dose of Antitoxin.*—Guinea Pig 4, weight 660 gm., had received 1.6 cc. of antitoxin subcutaneously. 4 cc. of toxin were injected into the jugular vein. This animal developed symptoms of intoxication 2 hours after the injection and died at the expiration of 4 hours. The autopsy findings were characteristic.

Guinea Pig 5, weight 510 gm., had received 1.5 cc. of antitoxin subcutaneously. 2 cc. of toxin were given intravenously. No immediate or delayed symptoms arose, and the guinea pig was in perfect health 3 weeks later.

Guinea Pig 6, weight 770 gm., normal control. 0.25 cc. of toxin was injected into the jugular vein. The guinea pig was found dead 16 hours later. The autopsy findings were typical.

*Experiment 3. 7 Days after the Prophylactic Dose of Antitoxin.*—Guinea Pig 7, weight 670 gm., had received 1.7 cc. of antitoxin subcutaneously. 2 cc. of toxin were given intravenously. No symptoms developed, and the guinea pig was normal 3 weeks later.

Guinea Pig 8, weight 600 gm., normal control. 1 cc. of toxin was injected into the jugular vein. The guinea pig died 3½ hours later, and characteristic lesions were present.

*Experiment 4. 9 Days after the Prophylactic Dose of Antitoxin.*—Guinea Pig 9, weight 650 gm., had received 1.6 cc. of antitoxin subcutaneously. 2 cc. of toxin were given intravenously. No symptoms had appeared 6 hours later, but the guinea pig was found dead 24 hours after the toxin was given. The autopsy findings were characteristic of *B. welchii* intoxication.

Guinea Pig 10, weight 650 gm., had received 1.6 cc. of antitoxin subcutaneously. 1.5 cc. of toxin were injected into the jugular vein. No symptoms of intoxication arose, and the guinea pig remained normal.

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<sup>3</sup> Differences in the manner of action of *B. welchii* toxin when administered by intravenous and subcutaneous routes are described in the paper already referred to.<sup>1</sup>

Guinea Pig 11, weight 700 gm., normal control. 0.7 cc. of toxin was given intravenously. Death occurred 4 hours later, and the lesions were typical.

*Experiment 5. 12 Days after the Prophylactic Dose of Antitoxin.*—Guinea Pig 12, weight 640 gm., had received 1.6 cc. of antitoxin subcutaneously. 0.7 cc. of toxin was injected into the jugular vein. No symptoms had arisen 8 hours later. The guinea pig was found dead 22 hours after the toxin was given, and the autopsy findings were typical.

Guinea Pig 13, weight 660 gm., had received 1.7 cc. of antitoxin subcutaneously. 0.5 cc. of toxin was injected into the jugular vein. The guinea pig remained normal.

Guinea Pig 14, weight 600 gm., normal control. 0.28 cc. of toxin was given intravenously. This guinea pig was found dead 20 hours later. The autopsy findings were typical.

*Experiment 6. 14 Days after the Prophylactic Dose of Antitoxin.*—Guinea Pig 15, weight 570 gm., had received 1.5 cc. of antitoxin subcutaneously. 0.25 cc. of toxin was injected into the jugular vein. Death occurred 20 hours later. Characteristic toxin lesions were found at autopsy.

Guinea Pig 16, weight 580 gm., had received 1.5 cc. of antitoxin subcutaneously. 0.25 cc. of toxin was given intravenously. No symptoms developed, and the guinea pig was in perfect health 3 weeks later.

Guinea Pig 17, weight 600 gm., normal control. 0.27 cc. of toxin was injected into the jugular vein. The animal was found dead 20 hours after the toxin was given, and typical lesions were present.

The foregoing experiments show that a passive immunity to *Bacillus welchii* toxin can be conferred on guinea pigs by the administration of the antitoxin. In these instances the immunity persisted for about 2 weeks. The protection from the toxin was at first very pronounced, thirty-two acutely lethal doses being harmless when injected directly into the blood stream (Experiment 1, Guinea Pig 2) 2 days after the antitoxin was given. 5 days after the prophylactic administration of the antitoxin, eight lethal doses were still harmless (Experiment 2, Guinea Pig 5), but sixteen lethal doses killed. The immunity to the toxin gradually decreased and was about exhausted at the end of 2 weeks. At this time some of the prophylactic animals were still somewhat more resistant to the toxin than normal animals, while others manifested no increased resistance (Experiment 6, Guinea Pigs 15, 16, and 17).

The fact that animals can be passively immunized to the toxin may in itself be of considerable practical significance; but when it is remembered that the toxin is a powerful aggressin, preparing the field

for bacterial multiplication, its high significance becomes at once apparent. The next series of experiments further emphasizes the importance of the part played by the toxin in infection by the bacilli and shows conclusively that large numbers of highly virulent bacilli are practically harmless when deprived of their toxin.

*Prophylaxis against Infection.*

A series of guinea pigs, ranging in weight from 250 to 350 gm., was given subcutaneously on the inner aspect of one hind leg 1 cc. of antitoxin per 100 gm. of weight. The resistance of the prophylactic series to infection with fresh cultures of the virulent bacilli was compared with that of normal guinea pigs of the same size. The protected guinea pigs were infected subcutaneously in the leg opposite to the one in which they had received the antitoxin. The quantity of culture given the individual animals was calculated on the basis of body weight, a certain fraction of a cubic centimeter being given for each 100 gm. of weight. The following protocols illustrate the results:

*Experiment 7. 24 Hours after the Prophylactic Dose of Antitoxin.*—Guinea Pig 18, weight 280 gm., had received 2.8 cc. of antitoxin. 0.28 cc. of culture was given subcutaneously. 7 hours later the infected leg was moderately swollen and stiff; no local crepitation and no symptoms of general intoxication. 24 hours after the inoculation the local swelling had almost disappeared, and the guinea pig was apparently well.

Guinea Pig 19, weight 280 gm., had received 2.8 cc. of antitoxin and was given 0.5 cc. of culture. 7 hours later the infected leg was swollen and stiff; crepitation could not be elicited, and there was no general intoxication. The next day the swelling and stiffness were subsiding, the guinea pig was active, and no extension of the infection arose.

Guinea Pig 20, weight 250 gm., had received 2.5 cc. of antitoxin and was given 0.9 cc. of culture. The results were identical with those of the two preceding animals.

Guinea Pig 21, weight 310 gm., normal control. 0.006 cc. of culture was given subcutaneously. This guinea pig was found dead 22 hours after it was inoculated. Autopsy revealed edema, gas, and disorganization of tissue, lesions typical of *B. welchii* infection.

In Experiment 7 it is shown that 50, 83, and 150 lethal doses of culture respectively failed to infect the guinea pigs which had re-

ceived a protective injection of antitoxin 24 hours previously. As 150 lethal doses was the largest quantity of culture given the height of the resistance to infection was not accurately determined.

*Experiment 8. 3 Days after the Prophylactic Dose of Antitoxin.*—Guinea Pig 22, weight 250 gm., had received 2.5 cc. of antitoxin and was given 0.4 cc. of culture. No symptoms developed aside from local swelling and stiffness which rapidly subsided. No evidence of multiplication of the bacilli.

Guinea Pig 23, weight 310 gm., had received 3.1 cc. of antitoxin. 0.6 cc. of culture was injected into the opposite leg. The symptoms and results were the same as in the preceding animal.

Guinea Pig 24, weight 300 gm., normal control. 0.004 cc. of culture was given subcutaneously. The animal died 22 hours later. Lesions of *B. welchii* infection were present at autopsy.

In this experiment the limit of the resistance to infection was again not reached, although one of the guinea pigs also received 150 lethal doses of the culture.

*Experiment 9. 5 Days after the Antitoxin.*—Guinea Pig 25, weight 260 gm., had received 2.6 cc. of antitoxin. 1.5 cc. of culture were injected into the opposite leg. Infection did not develop, and the local swelling and stiffness which always follow these inoculations rapidly subsided.

Guinea Pig 26, weight 280 gm., had received 2.8 cc. of antitoxin. 2 cc. of culture were given. Infection developed, and the guinea pig died 30 hours after inoculation. Lesions typical of *B. welchii* infection were present at autopsy.

Guinea Pig 27, weight 290 gm., normal control. 0.005 cc. of culture was injected subcutaneously. The guinea pig was found dead 20 hours later and showed typical lesions.

This experiment indicates that a protected guinea pig could resist 300 but not 400 lethal doses of the culture 5 days after the antitoxin had been given.

*Experiment 10. 8 Days after the Antitoxin.*—Guinea Pig 28, weight 260 gm., had received 2.6 cc. of antitoxin. 0.3 cc. of culture was given subcutaneously. The usual local swelling and stiffness followed, but no infection arose.

Guinea Pig 29, weight 280 gm., had received 2.8 cc. of antitoxin subcutaneously. 0.5 cc. of culture caused a fatal infection, with characteristic lesions.

Guinea Pig 30, weight 275 gm., normal control. 0.005 cc. of culture was given subcutaneously. Infection developed, and the guinea pig died 23 hours after inoculation.

This experiment shows that 60 lethal doses of culture failed to infect 8 days after the antitoxin had been administered. The resistance of the protected guinea pigs was tested again on the 11th day, and twenty lethal doses were not infectious. On the 14th and 15th days, however, the antitoxin-treated guinea pigs proved susceptible to infection, but whether to the same degree as the normal controls the experiment does not indicate. We may, however, assume that the antitoxin becomes greatly reduced in quantity from the 12th to 13th day after its administration.

*Therapeutic Property of the Antitoxin.*

We may now consider the effects which the antitoxin exerts upon established infection with *Bacillus welchii*. For this purpose guinea pigs weighing from 500 to 600 gm. were used, smaller ones being so susceptible to the infection that there is little time afforded for treatment. The amount of a standard culture that would infect and kill all of a series of guinea pigs between 30 and 48 hours was first determined. The infecting dose was calculated on the basis of body weight, and it was found that about 0.035 cc. of culture per 100 gm. of weight was effective within the time limit mentioned in all the animals inoculated ranging in weight from 500 to 600 gm. The procedure was to infect a number of animals at one time with the same culture and allow 24 hours to elapse before beginning treatment. At this time the condition of the animals was noted, especially the extent of the local lesions and the symptoms of general intoxication. One of the animals was etherized and autopsied, and to one or more the antitoxin was administered, while the others were left as untreated controls. Illustrative protocols follow:

*Experiment 11.*—Guinea Pig 31, weight 510 gm. 0.18 cc. of culture was injected into the muscles on the inner aspect of the hind leg. 24 hours after inoculation, a gaseous phlegmon involving the infected leg and the adjacent abdominal wall had developed, crepitation was readily elicited, and the guinea pig exhibited symptoms of severe intoxication—rough coat, rapid respiration, and drowsiness. 3 cc. of antitoxin were injected into the jugular vein. The next day the local lesion was subsiding, crepitation could not be elicited, the toxic symptoms had disappeared, and the guinea pig ate greedily. 3 cc. of antitoxin were given sub-

cutaneously to prevent a recrudescence of the infection. The general condition improved from day to day, while the infected leg became gangrenous, sloughed, and was infected with pyogenic organisms. Healing finally occurred, but the scar tissue caused deformity of the leg.

Guinea Pig 32, weight 560 gm. 0.2 cc. of culture was injected into the muscle on the inner aspect of the hind leg. 24 hours after inoculation there were swelling and edema, crepitation, and intoxication. The animal was not treated, and it died exactly 48 hours after inoculation. Autopsy revealed the characteristic lesions of *B. welchii* infection.

Guinea Pig 33, weight 580 gm. 0.2 cc. of culture was injected in the same manner as in Guinea Pigs 31 and 32. 24 hours after inoculation, the guinea pig was etherized and autopsied. There was a large gaseous phlegmon involving the infected leg and adjacent abdominal wall. The muscles of this region were pulpified and laden with bacilli. The opposite groin contained a gelatinous, serosanguineous exudate. The muscles of the abdominal wall and diaphragm were of pinkish hue. The lungs were pink and edematous.

Guinea Pig 34, weight 605 gm., was inoculated with 0.21 cc. of culture. This animal served as an untreated control and died at the end of 40 hours of typical *B. welchii* infection.

Experiment 11 was repeated a number of times, and it was found that the infection could be regularly arrested by the antitoxin after it was well established and extensive destruction of the tissues in the region of the infection had already taken place. Symptoms of severe general intoxication were always present at this stage of the disease. The effect of the antitoxin was often noticeable within from 30 minutes to 1 hour after its administration. The treated guinea pigs would become more active, their coats smooth, and they would be attracted by food, while the controls remained crouched in a corner, with heads down and coats rough, and could not be induced to eat.

#### DISCUSSION.

The experimental results here reported with the preventive and therapeutic applications of the antitoxin are highly suggestive. They derive significance from the fact that *Bacillus welchii* infections in guinea pigs and other susceptible animals<sup>4</sup> are comparable with in-

<sup>4</sup> Protection and curative experiments have been carried out also with the more highly susceptible pigeon. They confirm the results with guinea pigs with certain variations due to the difference in species.

fections with this organism in man. The experimental infections in the guinea pig differ, however, from the natural infection in man in two important points: (1) man possesses a higher natural resistance to infection; (2) the guinea pigs were infected with fresh virulent cultures, while man must, in the great majority of instances, derive infection from spores. Moreover, the protected guinea pigs were given many lethal doses of the living cultures. Such massive inoculations do not occur in man. It may therefore be safely predicted that man will not develop the infection as long as his body fluids and tissues contain adequate quantities of the antitoxin.

The possibilities of this passive serum protection has natural limits of time, depending upon the rapidity of elimination of the foreign serum. The experimental data presented in this paper, which agree with the experience with antidiphtheritic and antitetanic antitoxins, indicate that, in all probability, a passive immunity to *Bacillus welchii* infection of at least 2 weeks' duration can be conferred upon man by a single injection of the antitoxin. This immunity will be sufficient in the majority of instances, since only sporadic cases of *Bacillus welchii* infection arise later than the 10th day after injury, and the greater number occurs within 48 hours of that time.

In the light of the results obtained in treating the infection in guinea pigs, it is reasonable to hope that the antitoxin will be of value also as a therapeutic agent. The indications are that early infectious cases can be readily arrested and the more advanced and severe ones ameliorated, if not wholly checked, so that surgical interference may be resorted to with greater probability of effectiveness.

The antitoxin in man should be administered intravenously and probably locally, about the wound, as well.

#### SUMMARY.

1. It has been possible to confer on guinea pigs a passive immunity of about 2 weeks' duration to *Bacillus welchii* toxin through a protective administration of the antitoxin.
2. Guinea pigs which had received a prophylactic dose of *Bacillus welchii* antitoxin exhibited pronounced resistance to infection with the virulent bacilli for a period of 12 days.



3. Established infections in guinea pigs with *Bacillus welchii* have been arrested and controlled by treatment with the antitoxin.

4. The opinion has been expressed that it will be possible to prevent *Bacillus welchii* infection in man through the prophylactic use of the antitoxin and developed cases of the infection may be controlled by therapeutic injections of the same agent.