# COMPARISON BETWEEN THE SUBCUTANEOUS AND INTRACUTANEOUS METHODS OF TESTING THE VIRULENCE OF DIPHTHERIA BACILLI *

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For final diagnosis no method exists, other than that of animal inoculation, for differentiating between virulent diphtheria bacilli and nonvirulent diphtheria-like organisms. In diagnostic laboratories this fact is of economic importance on account of the number of guinea-pigs that must be used. The method usually employed is to isolate the organism, grow it for 48 hours in ascitic broth, and inject 1-2 c.c. of the culture subcutaneously into a guinea-pig. A control pig is injected with the same amount of the broth culture and also a protecting amount of antitoxin. If the organism is the diphtheria bacillus, the test pig will die within 2-3 days and the control pig will live. On necropsy, the test pig will show the typical lesions, that is, subcutaneous infiltration at the site of injection and hemorrhagic suprarenals. On the whole, this method gives satisfactory results, but it necessitates the use of 2 pigs for each test.

Recently, Zingher[1] has employed a method (suggested by the Neisser intracutaneous test) whereby 2 pigs may be used for 4-6 tests; the total time necessary to carry through a test being 4-6 days, as compared to 5-7 days for the subcutaneous method. This method, therefore, effects a saving both in time and animals.

Neisser recommended that the virulence of cultures be tested in the following way:

> One loopful of a 24-hour Loeffler slant of the organism is suspended in 1 c.c., 10 c.c., and 100 c.c. of physiologic sodium chlorid solution, and 0.1 c.c. of each suspension is injected intracutaneously on the abdominal surface of a guinea-pig. True diphtheria bacilli will incite a local inflammatory lesion with superficial necrosis in 48-72 hours, the intensity of the reaction depending on the number of injected organisms and their virulence. As a control, antitoxin containing 8 units per c.c., is added to an equal volume of the heaviest sus-

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[1] Proc. New York Path. Soc., 1915, 15, p. 18.

pension, and 0.1 c.c. of the mixture is injected intracutaneously into the same guinea-pig. The skin at the site of the control injection should remain normal in appearance.

Zingher found the use of 1 pig only for the test and control unsatisfactory, in that the direct addition of antitoxin in the control injection so immunized the animals that the test lesions were affected to a considerable degree. Further, when the amount of antitoxin was diminished to avoid the general immunization, the local action of the bacteria was not completely inhibited and lesions were found in both test and control areas. Zingher modified the technic as follows:

Two guinea-pigs are used for testing 4-6 strains. The hair on the abdominal surface is removed and 4 or 6 areas are marked according to the size of the pig. One pig serves as a control and receives about 100 units of antitoxin intracardially at the time of making the tests, or intraperitoneally 24 hours before. A bacterial suspension is made from a fresh 24-hour growth from an ordinary Loeffler's slant suspended in 20 c.c. of physiologic sodium chlorid solution; 0.15 of this emulsion is injected intracutaneously into both pigs. A very fine steel or platinum iridium needle should be used. If the injection is properly made, a circumscribed elevation appears which persists 1 or 2 minutes. Virulent strains induce a definite local inflammatory lesion which shows a superficial necrosis in 48-72 hours. In the control pig the skin remains normal. With nonvirulent strains no lesion will be found in either control or test animals.

In order to compare the value of the 2 methods of testing the virulence of diphtheria bacilli, 37 organisms were isolated from the throats of positive or suspected cases.

For the subcutaneous tests, the organisms were grown in meat infusion broth (neutral to phenolphthalein) to which 10% horse serum was added. After an incubation period of 48 hours, 2 c.c. of this toxin culture were injected into a test pig. A control pig received the same amount of culture and at the same time 100 units of antitoxin. Zingher's modification was used for the intracutaneous tests.

Twenty-two of the 37 cultures gave identical results by both methods. Fourteen of these were virulent and 8 were nonvirulent. In the virulent cases, the test guinea-pigs which were injected intracutaneously developed necrosis at the site of inoculation, while the control pigs showed no reaction. Those injected with toxin subcutaneously died within 3 days and the corresponding control pigs remained unaffected.

At this time a number of animals were lost because of an infectious disease that spread among them. This necessitated the repetition of 15 tests. A possibility of error in the subcutaneous method was

TABLE 1

RESULTS OF TESTS OF 37 CULTURES BY INTRACUTANEOUS AND SUBCUTANEOUS METHODS

| No. | Virulent Strains | | No. | Nonvirulent Strains | |
|---|---|---|---|---|---|
| | Intracutaneous Method | Subcutaneous Method | | Intracutaneous Method | Subcutaneous Method |
| 1 | Inflammatory infiltration | Test pig died | 1 | No necrosis | Test pig did not die |
| 2 | Necrosis | Test pig died | 2 | No necrosis | Test pig did not die |
| 3 | Necrosis | Test pig died | 3 | No necrosis | Test pig did not die |
| 4 | Necrosis | Test pig died | 4 | No necrosis | Test pig did not die |
| 5 | Necrosis | Test pig died | 5 | No necrosis | Test pig did not die |
| 6 | Necrosis | Test pig died | 6 | No necrosis | Test pig did not die |
| 7 | Necrosis | Test pig died | 7 | No necrosis | Test pig did not die |
| 8 | Necrosis | Test pig died | 8 | No necrosis | Test pig did not die |
| 9 | Necrosis | Test pig died | 9 | No necrosis | Test pig did not die |
| 10 | Necrosis | Test pig died | 10 | No necrosis | Test pig did not die |
| 11 | Necrosis | Test pig died | 11 | No necrosis | Test pig did not die |
| 12 | Slight necrosis | Test pig died | 12 | No necrosis | Test pig died† |
| 13 | Slight necrosis | Test pig died | * | No necrosis | Test pig did not die |
| 14 | Necrosis | Test pig died | 13 | No necrosis | Test pig died† |
| 15 | Necrosis | Test pig died | * | ..... | Test pig did not die |
| 16 | Necrosis | Test pig died | 14 | No necrosis | Test pig died† |
| 17 | Necrosis | Test pig died | * | No necrosis | Test pig did not die |
| 18 | Necrosis | Test pig died | | | |
| 19 | Necrosis | Test pig died | | | |
| 20 | Necrosis | Test pig died | | | |
| 21 | Necrosis | Test pig did not die | | | |
| * | Necrosis | Test pig died | | | |
| 22 | Necrosis | Test pig did not die | | | |
| * | Necrosis | Test pig died | | | |
| 23 | No necrosis | Test pig died | | | |
| * | Necrosis | Test pig died | | | |

* Second test. † Death due to an infectious disease.

brought to light as a result. In 3 cases, the test pigs died while the control pigs lived. This would undoubtedly have led to the conclusion that the control pigs were protected by the antitoxin received, and that the organism injected was the true diphtheria bacillus, had not the test pigs injected intracutaneously showed no lesions.

Necropsy findings in pigs dying from diphtheria were not always constant. The suprarenal bodies showed varying degrees of congestion. In 1 case the diphtheria bacillus was isolated from the heart's blood but the suprarenal bodies were apparently normal.

Variations in the susceptibility of the different animals used appeared to influence the results in a small degree. In 2 instances the test pigs inoculated subcutaneously did not react, while the test pigs inoculated intracutaneously showed marked necrosis. A repetition of the tests gave conclusive evidence that the organisms injected were virulent and that the failure to produce death was in all probability due to a certain amount of natural antitoxin possessed by the individual guinea-pigs.

In the other instance the 1st test pig injected intracutaneously was apparently insusceptible.

From the number of cultures tested, the intracutaneous appears to give more accurate results than the subcutaneous method and has a further advantage of being more economical in time and animals. A possible disadvantage lies in the fact that if 1 toxin pig dies, 4 or 6 tests must be repeated.