

**The virulence of diphtheria-like organisms : a note on the intracutaneous test / by A.J. Eagleton and E.M. Baxter.**

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## THE VIRULENCE OF DIPHTHERIA-LIKE ORGANISMS.

### A NOTE ON THE INTRACUTANEOUS TEST.\*

BY

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AND

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For testing the virulence of diphtheria-like organisms, Ingber and Soletsky<sup>1</sup> described a method of intracutaneous injection which was a modification of that originally described by Neisser. They advocated the method on the grounds of economy, and stated that in 10 cases it gave the same result as the subcutaneous method. These authors usually tested four strains on each pair of guinea-pigs.

We have been using a similar test for some months, and are submitting our experience of it in the hope that it may prove useful to the many public health pathologists in England who are working in the same field. The modifications we have introduced are as follows:

1. Standardization of inoculum by opacity.
2. Inclusion of a known virulent strain in each test.
3. Injection of control animal on the preceding day.
4. Administration to the unprotected animal of a "following dose" of antitoxin of such a size that the skin reactions are not obscured while the life of the guinea-pig is saved.

The technique of the test is as follows:

Two guinea-pigs are used for each test; both must be partially site.

*Protecting Dose.*—One animal is given 500 units of diphtheria antitoxin by intraperitoneal injection on the day preceding the test. This animal acts as a control.

*Preparation of Inoculum.*—The test strains and a known virulent diphtheria bacillus are grown eighteen hours on effler slopes, emulsified in saline, diluted and standardized to opacity so that the suspension contains approximately fifty million organisms per cubic centimetre.

\* From the Wellcome Physiological Research Laboratories.

*Injection of Suspensions.*—White-haired portions of the guinea pigs are depilated with calcium sulphide paste, and into corresponding skin areas of the two animals is injected 0.2 c.cm. of each suspension. The injections are strictly intradermal and at least half an inch apart. On each pair of animals we usually test eight or ten strains, including the control virulent.

*Following Dose.*—Four or five hours later the animal which was not given a protecting dose on the previous day receives 125 units of diphtheria antitoxin intraperitoneally.

*Readings* are taken for the next three days.

In the case of a virulent diphtheria bacillus the control animal shows nothing or a faint transient flush; the other animal shows a definite rose-red swelling which becomes more marked at each successive reading, and may terminate in slight necrosis. An avirulent diphtheria-like organism gives a negative result with both animals. If the test culture is contaminated with streptococci the result may be obscured by a reaction in both animals, but this does not always happen.

The advantages of the test are:

1. Economy of animals, as both survive.
2. Owing to the variability of growth shown by different strains, standardization by opacity is preferable to the use of a certain proportion of a slope culture (as in the American method).
3. Reliability. We have had the opportunity of comparing only a limited number of strains by the subcutaneous injection method; so far the agreement is satisfactory.

#### REFERENCE.

- <sup>1</sup> Zingher and Soleisky: *Journal of Infectious Diseases*, vol. xvii, No. 3, 1915.