

The virulence of diphtheria-like organisms : (further note) / by A.J. Eagleton and E.M. Baxter.

Contributors

Eagleton, A. J.

Baxter, E. M.

Wellcome Physiological Research Laboratories.

Publication/Creation

[Place of publication not identified] : [publisher not identified], [1922?]

Persistent URL

<https://wellcomecollection.org/works/vdawby9h>



Wellcome Collection
183 Euston Road
London NW1 2BE UK
T +44 (0)20 7611 8722
E library@wellcomecollection.org
<https://wellcomecollection.org>

THE VIRULENCE OF DIPHTHERIA-LIKE ORGANISMS.

(Further Note.)

BY

A. J. EAGLETON, M.D.LOND., M.R.C.P.LOND.,

AND

E. M. BAXTER, M.Sc. SHEFFIELD.

(From the Wellcome Physiological Research Laboratories.)

THE bacteriological diagnosis of diphtheria, though based generally on the morphology and cultural characteristics of the *Bacillus diphtheriae*, must depend ultimately on the animal test, whereby the pathogenicity of the infecting organism can be definitely established. While the animal test is advisable, even in clinical work, as a periodical check on the routine methods of diagnosis, in dealing with the carrier problem and prevention of diphtheria it is indispensable. The time may come when some other test may replace it; this is, however, very improbable.

By the intracutaneous method (Eagleton and Baxter, 1921) a large number of pathogenicity tests can be carried out speedily and economically. Further experience of this method has served to confirm our belief in its reliability, evidence of which is given in the second part of this paper. The first section deals with 161 cultures of diphtheria-like organisms, all of which were tested for pathogenicity, and the majority for fermentative power. Slopes of Loeffler's serum medium were employed for primary cultures, and plates of the same medium for subsequent purification.

The intracutaneous method was used for testing pathogenicity, checked frequently, especially when the findings were negative, by subcutaneous injections, as will be described later.

I. EXAMINATION OF CULTURES.

The cultures were derived from three sources, and each group will be considered separately.

(a) Thirty-seven cultures from cases of clinical diphtheria gave the following results:

Morphology.	Glucose.	Saccharose.	Virulence.
<i>B. diphtheriae</i>	Acid	Negative	+ 33
<i>B. diphtheriae</i>	Not done	Not done	+ 3
Hoffmann's bacillus ...	Not done	Not done	- 1
			37

Of the 33 virulent cultures of *B. diphtheriae* the "sugar" reactions of which were tested, 2 fermented saccharose as well as glucose. These cultures, after plating, had been maintained on Loeffler medium for over ten weeks, and were seemingly pure, judging by smear preparations and appearance of growth. On replating, however, a very slight contamination was found—in one case a coccus and in the other a Gram-positive bacillus which produced acid in saccharose. When freed from these impurities the two strains failed to ferment saccharose.

(b) A single throat swab was taken from 112 patients who had had diphtheria and had completely recovered at the time of examination. Nineteen strains of *B. diphtheriae* and Hoffmann's bacillus were obtained, and gave the following results :

Morphology.	Glucose.	Saccharose.	Virulence.
<i>B. diphtheriae</i>	Acid	Negative	+ 9
<i>B. diphtheriae</i>	Not done	Not done	+ 1
<i>B. diphtheriae</i>	Acid	Negative	— 1
Hoffmann's bacillus ...	Negative	Negative	— 5
Hoffmann's bacillus ...	Not done	Not done	— 3
			19

Five of the nine virulent strains, when first tested in sugars, produced acid in saccharose; this was, however, due to a very slight degree of contamination. The fermentation of saccharose in these cases was generally slight, and might not take place till after the third day of incubation.

The most difficult contamination to eliminate is a Gram-positive sporing bacillus, which may lie dormant for weeks in a culture on solid medium. This organism ferments glucose and saccharose, and, when abundant or in pure culture, liquefies Loeffler medium.

(c) Comprises 105 cultures from 67 carriers, none of whom had had diphtheria, but most of whom had been in contact with a case. The results found in this group were as follows :

Morphology.	Glucose.	Saccharose.	Virulence.
<i>B. diphtheriae</i>	Acid	Negative	+ 30
<i>B. diphtheriae</i>	Not done	Not done	+ 3
<i>B. diphtheriae</i>	Acid	Negative	— 52
<i>B. diphtheriae</i>	Acid	Acid	— 8
<i>B. diphtheriae</i>	Not done	Not done	— 1
Hoffmann's bacillus ...	Negative	Negative	— 11
			105

Of the 30 virulent cultures, one produced acid in saccharose, but as with similar cases in the other groups, a very slight contamination was found to be responsible.

When we compare these three groups we notice that in Group A only one culture was avirulent and that was Hoffmann's bacillus. All the remainder were virulent, and,

except for three not tested, gave typical "sugar" reactions. In Group B we find 10 virulent and one avirulent *B. diphtheriae*, the remainder being Hoffmann's bacillus; whereas in Group C, of 94 bacilli morphologically resembling *B. diphtheriae*, only 33 were virulent; excluding the eight strains which fermented saccharose (*B. xerosis*) and the one avirulent strain, the "sugar" reactions of which were not tested, we find 33 virulent and 52 avirulent.

Of the cultures of *B. diphtheriae* from clinical cases we find 100 per cent. virulent; of those from convalescents 90.9 per cent.; while of the cultures from carriers which were typical on morphology and "sugar" reactions we find only 38.8 per cent. virulent.

These results agree with the findings of most other workers in so far as they show that avirulent strains are rarely found except in people who have never, or at all events not recently, suffered from diphtheria (Graham Smith, 1908, Arkwright, 1912).

Fermentation Reactions.—Of the 161 cultures in the three groups the sugar reactions were tested in all except twelve instances. We used as a routine only glucose and saccharose. Thirty strains were also examined for action on dextrin, salicin, and litmus milk. We found, however, that by using glucose and saccharose alone we obtained as much information as we could from the more extended set of reagents. All the virulent *B. diphtheriae* which we have tested hitherto produce acid in glucose but not in saccharose. The same reaction was, however, given by 53 out of 61 avirulent cultures, though the amount of acid produced by two of them was very slight. We have not so far met with a virulent strain which, when absolutely pure, produces acid in saccharose. Graham Smith (1908) has reported several from one epidemic. Our difficulties in getting rid of contamination by saccharose-fermenting organisms have been noticed. We succeeded only by repeated plating, and in more than one instance after picking off several colonies from the same plate and testing the sugar reactions of each, we found that some of the "single" colonies failed to ferment saccharose, whilst others did so.

We have so far found no evidence that a culture may "lose its virulence" on being kept. It need only be mentioned that smears must be made from every culture before it is used for inoculating animals. One culture in our series appeared to be a pure culture of Hoffmann's bacillus and was avirulent by intracutaneous test. Two months later, when examined for morphology, sugar reactions and virulence, it was found to be indistinguishable from *B. diphtheriae* on morphology and "sugar" reactions, and to be virulent. The culture was plated, and from the plate a number of colonies were picked off. Some proved to be pure cultures of Hoffmann's bacillus, others *B. diphtheriae*. Hoffmann's bacillus and *B. diphtheriae* may therefore live in the same tube for months and neither be destroyed. A culture originally avirulent may, when next examined, be found virulent because the *B. diphtheriae* are in enormous excess, and vice versa.

II. RELIABILITY OF INTRACUTANEOUS TEST.

We have compared the results of determination of virulence by the intracutaneous method with those obtained by the

THE UNIVERSITY OF CHICAGO
LIBRARY

100 EAST 57TH STREET
NEW YORK 22, N. Y.

1950

1950

1950

1950

1950

1950

1950

1950