ON THE PRODUCTION OF ANTITOXIN BY THE INJECTION OF FILTRATES OF CULTURES OF NON-VIRULENT DIPHTHERIA BACILLI.

By J. A. ARKWRIGHT, M.D.

(*From the Lister Institute of Preventive Medicine.*)

We are still in doubt regarding the exact position of those bacilli which, morphologically and culturally, appear to be identical with the Klebs-Loeffler bacillus but which differ from it only in the absence of specific pathogenic power for laboratory animals. Most writers regard such non-virulent strains as true diphtheria bacilli which have lost their virulence and toxigenic power either temporarily or permanently. Others however are inclined to place them in a group allied to that of the genuine Klebs-Loeffler bacillus and express doubts as to whether they at any time possessed a claim to pathological significance (Graham-Smith, 1904).

The experiments detailed in this brief communication were made in the course of an attempt to demonstrate the presence of small quantities of specific diphtheria-toxin in broth culture filtrates of these non-virulent strains.

Three non-virulent strains were employed. They were all isolated from the fauces during an investigation of a diphtheria outbreak in a boys' school. Details of the bacteriological findings in connexion with this epidemic have been put on record by the writer (Arkwright, 1908).

Notes regarding the strains employed:

Strain "A" was recovered from the throat of a boy, who two months before had a sore throat and at the time of examination still had a nasal discharge. This strain was of the medium-long type.

Strain "B" was an extremely long and segmented form, while strain "C" was a rather short type. These two latter strains were isolated from boys who had not been observed to suffer from sore throat.

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In glucose-peptone water all three strains produced an acid reaction, but cane-sugar media were not altered.

The subcutaneous inoculation of large doses (e.g. 5 c.c.) of 2-day and 8-day old broth cultures, produced no illness in guinea-pigs and, when massive doses large enough to kill were given intraperitoneally, no protection or postponement of death was afforded by the preliminary or simultaneous injection of antitoxin.

Although all attempts to demonstrate by direct methods the presence of toxin in broth culture filtrates of these strains had failed, it was thought that the injection of these filtrates might lead to the development of antitoxin in the horse, even if only very minute quantities of toxin or toxoid were present. Similar experiments by Petrie (1905) were made with strains of Hofmann's bacillus, but with negative results.

Scheme of Inoculation and Serum-tests.

Before the commencement of the inoculations the horse was bled on 4th November 1907 and the normal antitoxin-content of its serum estimated. It was found to possess a quarter of a unit per cub. cent.

Filtrates of 9-day broth cultures of strain "A" were then inoculated in the following quantities at intervals of two or three days: 8th Nov. 1907, 0·5 c.c.; 11th Nov., 1 c.c.; 13th Nov., 5 c.c.; 15th Nov., 10 c.c.; 18th Nov., 30 c.c.; 20th Nov., 80 c.c.; 22nd Nov., 200 c.c.; 25th Nov., 400 c.c.; 27th Nov., 1000 c.c. On Dec. 7th (i.e. 10 days after the last injection) the horse was bled to the amount of six litres.

The serum was now found to contain 4 units per cub. cent.

After a rest, the horse received two large doses of filtrate from strain "B", viz. 24th Jan. 1908, 200 c.c.; and 27th Jan., 600 c.c.

On 11th Feb. 1908 it was again bled when the serum showed an antitoxin value of 25—30 units per cub. cent.

During March 1908 the horse received further injections of filtrate from strain "C" but no appreciable rise in potency resulted.

Conclusions.

(*a*) Filtered broth cultures of two strains, which were morphologically and culturally indistinguishable from virulent Klebs-Loeffler bacilli but which possessed no pathogenicity for guinea-pigs and apparently were non-toxigenic, led to the development of antitoxin when injected in large quantities into a horse.

One strain raised the potency of the serum from $\frac{1}{4}$th unit (possessed normally) to 4 units per cub. cent. Further treatment with a second strain caused a further rise to 25 units per cub. cent. Subsequent treatment with a third strain produced no further elevation of the antitoxin-content.

(*b*) These strains though completely non-virulent must be regarded as true *B. diphtheriae* in virtue of their power to excite the production of antitoxin in the horse.

(*c*) Whether these strains have lost their pathogenic properties permanently or only temporarily cannot be stated, but all attempts to raise their virulence or toxigenic power by guinea-pig passage or by cultural methods have so far failed.

Further experiments are in progress on the antitoxin-producing power of various non-virulent strains of *B. diphtheriae*.

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