On the identity of the toxins produced by serologically different strains of Bacillus diphtheriæ / by Percival Hartley.

Reprinted from THE LANCET, 1923, I., 17.

117

ON THE IDENTITY OF THE TOXINS PRODUCED BY SEROLOGICALLY DIFFERENT STRAINS OF *BACILLUS DIPHTHERIÆ*.

BY PERCIVAL HARTLEY, *M.C.*, D.SC. LOND.
(*From the Wellcome Physiological Research Laboratories.*)

HAVENS (1920) examined 206 strains of the diphtheria bacillus by means of a monovalent agglutinating serum and found that 169 strains (82 per cent.) were agglutinated by it in a dilution of 1/4860, and the strain used for the production of this serum was also agglutinated at this dilution. Thirty-seven strains (18 per cent.) failed to agglutinate. Accordingly a member of this smaller group was used for the production of a second agglutinating serum and it was found that all the members of this second group were agglutinated by this serum in a dilution of 1/4860. No evidence of cross agglutination was obtained; the two sera were specific for their respective groups. Havens also found that whereas the toxins produced by members of group 1 were neutralised by the antitoxin in common use (which is usually made from strain Park Williams No. 8 belonging to group 1) the toxins produced by the members of group 2 required very large amounts of antitoxin for their neutralisation. As a rule 20 units of standard antitoxin protected against one minimum lethal dose of group 2 toxin, while 5 to 10 units regularly failed to protect. Some samples of antitoxin, however, required to be used in much larger doses in order to neutralise one minimum lethal dose of group 2 toxin; in two cases 100 units were necessary, and in a third case even 1000 units failed to protect. Havens concluded from his experiments that standard antitoxin contains group antitoxin which protects against the toxins from both strains of the diphtheria bacillus, and that the amount of this group antitoxin, though never very large, varies in different samples.

Havens draws conclusions from his work which have a very important bearing on the preparation of diphtheria antitoxin and its practical use. He suggests that those cases which do not yield to treatment except by the use of very large amounts of antitoxin may be due to infection by organisms belonging to group 2. He also points out that, in spite of the increasing use of antitoxin and its administration early in the disease, the death-rate from diphtheria in America is still about 10 per cent., and this again may be due to infection by organisms belonging to group 2. Other procedures which, according to Havens, are affected by his results are the Schick test and active immunisation by toxin-antitoxin mixtures. As practical measures Havens recommends the inclusion of toxin from a member of group 2 in the routine preparation of antitoxin, the use of a mixture of toxins of both groups for the Schick test, and the use of mixed toxins and antitoxins for active immunisation.

Further Experiments.

Paxson and Redowitz (1922) repeated Havens's experiments and confirmed his results in so far as the occurrence of two serologically different groups of diphtheria bacilli are concerned. They were unable to obtain any evidence that the toxins from group 2 were not neutralised by standard antitoxin, and they concluded that antitoxin prepared from toxin obtained from group 1 strains neutralised equally well the toxins belonging to either group.

Park, Williams, and Mann (1922) examined five strains received from Durand (1920) who had shown by agglutination and absorption tests that they belonged to five different groups. Four strains belonging to Havens's group 2 were also examined. These workers fully corroborated Durand's results, and found that the four Havens's strains were not agglutinated by their type 1 serum, and they conclude "that in all communities there are many types and at least several dominant ones." They found, however, that the common antitoxin protected against living cultures of organisms belonging to five different groups, including three of Havens's group 2 strains and, further, that toxins prepared from members of all five groups were neutralised by the usual amounts of antitoxin.

The question of the identity of the toxin from different strains of the diphtheria bacillus and the efficacy of antitoxin arises from time to time in this country. It was stated in a recent report of the medical officer of health of one of our large cities that larger doses of antitoxin are required to-day to produce the same clinical improvement than were needed seven years ago, and it was suggested that this may be due to an alteration in the type or toxicity of the prevailing bacillus. A. S. G. Bell (1922), working at the Royal Army Medical College, investigated 130 strains of *B. diphtheriæ*, over 90 per cent. of which were isolated from swabs taken in the London area during a recent epidemic. An agglutinating serum prepared from strain Park Williams No. 8 agglutinated 17 (13 per cent.) of the strains. A second serum, made from a strain not agglutinated by the type 1 serum, agglutinated eight strains (6 per cent.), while a third serum, made from a strain which was not agglutinated by either type 1 or type 2 serum, agglutinated 80 strains (61 per cent.). Twenty-five strains (20 per cent.) were not agglutinated by either of the three sera prepared.

At Major Bell's request I undertook the study of the toxins produced by members of these three groups. The main object of the inquiry was to ascertain whether the toxins were neutralised by the antitoxin in common use which is made from the toxin produced by the Park Williams No. 8 strain.

Method of Present Test.

Fifteen strains, received at different times, were investigated. They were sown on 100 c.cm. quantities of the medium used in these laboratories for toxin production (1922, Hartley, P., and Hartley, O. M.) and after incubation at 37° C. for three days

0·5 per cent. of pure carbolic acid was added. The sterilised cultures were allowed to stand overnight in the dark and then filtered. Preliminary tests on the toxin were carried out by the intracutaneous method (Römer and Sames (1909); Glenny and Allen (1921)), the results of which showed that three of the strains had produced practically no toxin, that 12 had produced toxin in very variable amounts, and that all the toxins, irrespective of the group to which the strain belonged, were neutralised by the antitoxin in common use.

The results obtained by the intracutaneous method of testing indicated that eight of the toxins were of sufficient potency to admit of more complete examination by tests on the whole animal. Accordingly, the minimum lethal dose (M.L.D.), the dose which neutralised exactly one unit of antitoxin (Lo dose), and the dose which, when mixed with one unit of antitoxin, caused the death of the animal in 4–5 days (L+ dose) were determined in order to ascertain whether any toxin exhibited any unusual feature, and particularly whether the toxins from strains belonging to group 2 or group 3 required abnormally large amounts of antitoxin for their neutralisation.

The results of these experiments are summarised in the following table.

Tests of Toxin from Different Strains of B. diphtheriæ.

Strain.	Type.	M.L.D. in c.cm.	L+ dose. c.cm.	Lo dose. c.cm.	No. of M.L.D.'s neutralised by 1 unit of antitoxin.
79 I.	1	0·014	0·75	0·65	46
88	1	0·012	0·65	0·55	46
79 II.	1	0·045	2·90	2·25	50
Black.	2	0·05	2·30	1·80	36
Lister VII.	2	0·025	1·20	1·00	40
Swindon.	3	0·006	0·68	0·55	91
Phillips.	3	0·007	0·36	0·30	43
Evans.	3	0·009	0·48	0·38	42

Seven of these toxins, although varying widely in potency, are very similar in composition, and the eighth (from strain "Swindon") differs from the others only in so far as the proportion of toxoid is somewhat less. There is no evidence that any strain produces a toxin which requires more than the usual amount of antitoxin for neutralisation. Post-mortem examinations were carried out on all the animals which died and all showed lesions typical of diphtheria poisoning.

The three strains "Swindon," "Phillips," and "Evans," when grown on liquid medium, exhibited several interesting features. These strains all belong to group 3—the largest of Bell's groups. Recently isolated strains of *B. diphtheriæ* usually produce toxin of relatively low potency. Theobald Smith and Walker (1896) studied the toxins produced by 42 different strains and found that the minimum lethal dose varied from 0·036 c.cm. to 0·12 c.cm. G. Dean (1913) states that the minimum lethal dose of toxins from recently isolated strains ranges from 0·1 c.cm. to 0·02 c.cm. and that on rare occasions the dose may reach 0·01 c.cm. The minimum lethal dose of the strains "Swindon," "Phillips," and "Evans" was

0·006 c.cm., 0·007 c.cm., and 0·009 c.cm. respectively. These three strains were also exceptional in so far as they all grew readily as surface films, and not throughout the medium, and they all grew very rapidly. It was possible to compare them under similar experimental conditions with strain Park Williams No. 8, the behaviour of which on the medium used was well known. This strain produces a complete film in 20 to 24 hours, but under the same conditions strains "Swindon," "Phillips," and "Evans" produced a film of equal density in 12 to 14 hours. The film produced by strain Park Williams No. 8 remained on the surface almost indefinitely, while the film produced by these three strains frequently sank to the bottom of the vessel after 18 to 24 hours' incubation, and shortly afterwards a second film began to grow. It is unfortunate that more strains were not investigated, as it would have been interesting to see whether rapid growth and the production of relatively large amounts of toxin were characteristics common to all members of this particular group of diphtheria bacilli.

Results Obtained.

The results are in agreement with those obtained by Paxson and Redowitz and by Park, Williams, and Mann. Further, these results obtained experimentally in the laboratory are in accord with the vast amount of clinical evidence concerning the efficacy of monovalent diphtheria antitoxin which has been accumulated during the last 25 years all over the world. It is reasonable to suppose that if different strains of diphtheria bacilli produced toxins which exhibited wide differences in their affinity for antitoxin, evidence—or at least indications—of this would have been forthcoming from workers studying diphtheria from the clinical side. As Park, Williams, and Mann point out, ever since diphtheria antitoxin has been used in practice hundreds of thousands of persons known to be contacts have been given practically complete protection for two weeks by the injection of monovalent antitoxin; and, further, routine virulence tests throughout the world have shown that a monovalent antitoxin protects animals against a dose of living culture fatal to others not given antitoxin.

References.—Bell, A. S. G.: Journ. R.A.M.C., 1922, xxxviii., 48. Dean, G.: Nuttall and Graham Smith, The Bacteriology of Diphtheria, Cambridge Univ. Press, 1913, 462. Durand, P.: Compt. rend. de la Soc. de Biol., 1918, lxxxi., 1011; and 1920, lxxxiii., 611 and 612. Glenny, A. T., and Allen, K.: Journ. Path. and Bacteriol., 1921, xxiv., 61. Hartley, P., and Hartley, O. M.: Ibid., 1922, xxv., 458. Havens, L. C.: Journ. Infect. Dis., 1920, xxvi., 388. Park, W. H., Williams, A. W., and Mann, A. G.: Journ. Immunol., 1922, vii., 243. Paxson, W. H., and Redowitz, E.: Ibid., 1922, vii., 69. Römer, P. H., and Sames, Th.: Ztschr. f. Immunitätsforsch., 1909, iii., 344. Smith, Theobald, and Walker, E.: Twenty-eighth Annual Report of State Board of Health, Massachusetts, 1896, 649.

The Lancet Office,
1, Bedford Street, Strand, W.C.2