TYPES OF *C. DIPHTHERIAE* IN CAPE TOWN

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Reports from different areas in England and Scotland, and also from Berlin, have shown that the distribution of *C. diphtheriae* types varies considerably from one locality to another. It therefore seemed of interest to investigate their incidence in another country at a considerable distance from Europe. Strains from all diphtheria patients admitted to the Capetown City Hospital for Infectious Diseases were accordingly received from the Government Laboratory over a period of approximately seven months and investigated as to cultural characteristics, biochemical reactions, and virulence.

Methods

Cultures reported as positive for *C. diphtheriae* were subcultured on to Loeffler slopes, incubated for eighteen hours, and then plated on to McLeod's tellurite rabbit blood agar, the colonial appearance being examined after twenty-four and forty-eight hours. Pure cultures were then obtained by subculture to Loeffler slopes, which after eighteen hours' incubation were tested for virulence by the intracutaneous guinea-pig method, for fermentation of glucose and of saccharose, for fermentation of starch (Bacto) and of glycogen (B.D.H.) in Hiss's serum water, and for their type of growth in nutrient beef heart infusion broth after twenty-four and forty-eight hours' incubation.

Bacteriological Observations

Altogether 109 strains were isolated; all fermented glucose but not saccharose, and with one exception were virulent by intracutaneous guinea-pig test. They were classified according to the scheme introduced by H. A. Wright and M. H. Christison (1935) (Table I). An additional type (Type VII) was included. This will be discussed later.

TABLE I.—The Incidence of Biological Types of *C. diphtheriae* in Capetown

Type	Colony Form on McLeod's Medium	Fermentation of Starch	Virulence	Percentage Incidence
I	Mitis type	—	+	53.2
II	Intermediate type	—	+	0
III	Gravis type	+	+	6.4
IV	" "	—	+	22.0
V	" "	+	—	0.9
VI	" "	—	—	0
VII	Mitis type	+	+	12.9
Unclassified				4.6

Type I.—Of these 109 strains fifty-eight (53.2 per cent.) were classified in this group. Of these, forty-nine had typical black shining "mitis" colonies. Nine other strains differed from the typical mitis form only in that their colonies were slightly matt or very faintly striated. They were therefore not sufficiently different to classify as any other type. In broth all these strains produced cloudy growth without pellicle formation. In some instances, unrelated to the type of colony, the growth was slightly floccular, but was very easily broken up on gentle agitation.

Type II.—No strain fell into this group—the "intermediate" group of Anderson and his co-workers.

Type III.—Seven (6.4 per cent.) strains only were classifiable as Type III. They differed slightly in colonial appearance from the "gravis" strains described by Anderson and his co-workers in that the colonies were slightly smoother, slightly darker, and slightly smaller, but they were not sufficiently different to classify as any other type. In broth they all produced a pellicle, deposit, and fine granular type of growth.

Type IV.—Twenty-four (22 per cent.) strains fell into this group. They could be further subdivided by their colonial appearance; thus seventeen produced the same type of colony as those of Type III above, three produced the typical daisy-head gravis colony, and four gave a mixture of these two types of colony. In broth all these strains produced a pellicle, deposit, and a fine granular type of growth.

Type V.—One strain (0.9 per cent.) belonged to this group. It also had the slightly smoother type of gravis colony which predominated in Types III and IV. In broth it produced granular growth, pellicle, and deposit.

Type VI.—No strain fell into this group.

Type VII.—This type has been added, since fourteen strains (12.9 per cent.) differed from Type I only in that definite fermentation of starch and glycogen occurred during twenty-four hours' incubation. Eight of these strains showed typical mitis colonies and six only differed by having a slightly matt surface.

Unclassified.—Five (4.6 per cent.) strains were not classified owing to their type of growth in broth. Three were of the mitis type of colony, but one was granular in broth and fermented starch and glycogen, another produced a very slight pellicle and did not ferment starch or glycogen, and the third gave a granular growth, deposit, and pellicle, and fermented starch and glycogen.

Two strains produced granular growth and pellicle formation in broth, fermented starch and glycogen, but in colony form were not clearly differentiable as gravis or as mitis.

Clinical Observations

Clinical notes were obtained independently from the Capetown City Hospital and in a few instances from private practitioners. The cases were grouped as "severe," "moderately severe," and "mild" according to the classification of Bie (1929). Owing to the mildness of diphtheria in Capetown the additional group of "very mild" was added to include those cases in which slight sore throat or nasal discharge and pyrexia accompanied by a positive swab were found. In the "severe" group were included all those cases in which laryngeal involvement occurred, or in which evidence of toxicity was present, such as albuminuria and palatal paralysis.

During the period in which the strains were collected there was no epidemic of diphtheria in the locality. In the majority of the cases the disease was mild, and there was little toxicity. As has been previously observed in Capetown, the number of patients with laryngeal involvement was high, in this series being 7.3 per cent. The dosage of antitoxin was on the whole less in the mild cases than in the severe, being on an average 20,000 units in the very mild, 30,000 units in the mild, and 50,000 units in the moderately severe and severe cases. Usually it was given later in the disease in the mild (three and a half to four days) than in the severe (two and a half to three days) cases.

Clinical data were procured in ninety-six cases, those in which the cultures proved avirulent or the strains atypical being omitted from the series. Of these ninety-six cases sixty-seven (70 per cent.) were due to Types I and VII, and twenty-nine (30 per cent.) to Types III and IV, the distribution in relation to clinical severity being shown in Table II.

Of the fourteen severe cases three were fatal—that is, in this series there was a mortality of 3.1 per cent. Of the three fatal cases one was laryngeal, tracheotomy being

performed. The child, aged 4 years, was very toxic, and died in spite of the administration of 100,000 units of antitoxin. The strain in this case was Type IV. The other cases, due to Types I and VII respectively, were haemorrhagic and very toxic.

The severe cases include seven laryngeal, of which five were due to Type I and two to Type IV. Of the remaining four severe cases three had palatal paralysis and one

TABLE II.—*The Distribution of Types of C. diphtheriae in Relation to Clinical Severity*

Type of Case	Type I	Type VII	Total—Types I and VII	Type III	Type IV	Total—Types III and IV	Total Cases
Very mild	26	5	31 (76 %)	4	6	10 (24 %)	41
Mild	14	5	19 (70 %)	2	6	8 (30 %)	27
Moderately severe	8	0	8 (57 %)	0	6	6 (43 %)	14
Severe	10	1	11 (78 %)	0	3	3 (22 %)	14

was haemorrhagic and very toxic. It is thus seen that severe toxæmia was not common. There was no evidence of gravis strains being associated with more severe cases of diphtheria, but the small number of such cases rendered the figures too few for definite conclusions to be drawn.

The predominant strains of *C. diphtheriae* were found to be of Types I and VII (70 per cent.). The majority of the cases were clinically mild. These findings are in agreement with those of workers in other areas regarding the association of mitis strains with a mild type of diphtheria. It is interesting to note that no strain of Type II (intermediate) was found.

Discussion

In 1931 Anderson and his co-workers classified the Leeds strains of *C. diphtheriae* into three groups—"gravis," "mitis," and "intermediate." Only a small percentage failed to fall into these groups. Other workers have found aberrant strains ranging from 1.5 per cent. in Manchester (Robinson and Marshall, 1934) to 31.5 per cent. in Staffordshire (Menton, Cooper, Duke, and Fussell, 1933). The possibility therefore arises that the aberrant strains of one locality may be present in considerable numbers in another. The introduction by Wright and Christison (1935) of the numerical system of typing, with its possibility of expansion, adds considerably to the ease of classifying such strains. Thus of 109 strains isolated from clinical cases of diphtheria in Capetown only fifty-six could be clearly classified as mitis or as gravis, leaving fifty-three which would have to be classified as atypical under the scheme of the Leeds workers. Under the classification of Wright and Christison twenty-four of these strains could be placed in the Type IV group—the non-starch-fermenting, virulent gravis type. Such forms have been found in other areas only in small numbers—for example, 3.4 per cent. in Edinburgh (Wright and Christison, 1935). Of the remaining strains fourteen were placed in the newly introduced group of Type VII—mitis colony, starch-fermenting, and virulent.

Since the introduction by Anderson and his co-workers of the three types—gravis, mitis, and intermediate—the stability of their major characteristics has been questioned. Cultural characteristics in broth have been found an unreliable criterion. Regarding colonial form, Christison (1935) found that the three types underwent marked variation *in vitro*. She stated that gravis strains fermented starch irrespective of their colony structure, whereas the intermediate and mitis and their variants

failed to do so. She suggested that starch fermentation was a more reliable criterion than colony form for identifying the gravis type. Other workers have also maintained this position.

This view seems no longer tenable, since Wright and Christison (1935) found 14.3 per cent. of Edinburgh strains in Types IV and VI—that is, gravis in colony form, yet non-starch-fermenting. Others have included similar strains in their atypical group. Anderson and his co-workers (1933) report the loss of starch fermentation by one strain of gravis type on subculture, and recently Mair (1936) has found that the ability to ferment starch is not firmly fixed in some strains. It is therefore of interest to note that of the 109 strains isolated in Capetown 22 per cent. have been found to be of gravis type in colony form and growth in broth, yet not to ferment starch or glycogen, and 12.9 per cent. of the mitis type in colony form and growth in broth, yet to produce definite fermentation of starch and glycogen.

Summary

1. Of 109 strains of *C. diphtheriae* from clinical cases of diphtheria admitted to the Capetown City Hospital 53.2 per cent. were Type I; none of Type II; 6.4 per cent. Type III; 22 per cent. Type IV; 0.9 per cent. Type V; none of Type VI; and 4.6 per cent. unclassified. An additional type (Type VII) was introduced, since 12.9 per cent. were of mitis type in colony form and in growth in broth, but fermented starch and glycogen.

2. The mildness of diphtheria in Capetown was in agreement with the predominant strain being of the mitis type. The sparsity of severe cases of diphtheria made the figures too few to correlate gravis strains with the severity of the disease.

3. An interesting feature of the findings was the comparatively high percentage of strains of Type IV—namely, the non-starch-fermenting, virulent gravis type, and of Type VII, the starch-fermenting, virulent mitis type.

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R. W. Huntington, jun., and D. Wilkes-Weiss (*J. Pediat.*, October, 1936, p. 449) record their observations on fifty-four cases of influenzal meningitis admitted to the St. Louis Children's Hospital from 1918 to 1935, during which period there were 160 cases of meningococcus meningitis, seventy-four of streptococcus meningitis, sixty-two of pneumococcus meningitis, and twenty-two of staphylococcus meningitis admitted to the same hospital. Only sixteen were females and only twelve over 2 years of age, the oldest being 13 years and the youngest 2 months. Three of those who were over 2 years of age recovered. The onset was usually primary, but in ten the onset was preceded by otitis or pneumonia, and in others there was a history of trauma. The average duration of the disease was fourteen days. Serum treatment was used in fifteen cases, but only one recovered.