

AN ANALYSIS OF 510 STRAINS OF *CORYNE-  
BACTERIUM DIPHTHERIAE*.

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SINCE Anderson, Happold, McLeod and Thomson (1931) described their division of the varieties of *Corynebacterium diphtheriae* into three groups—*gravis*, intermediate and *mitis*—and submitted correlations with clinical types of diphtheria, several workers have published results of investigations which, for the most part, are against such a hard and fast classification.

The present communication aims at recording briefly an analysis of over 500 positive swabs which have passed through this laboratory during the past year. About 60 were from cases that have been under treatment in Belvidere Fever Hospital, about a dozen were from cases in similar institutions in Paisley and Motherwell and the remainder were sent in for routine diagnosis by general practitioners in the Glasgow area.

Colony appearances on McLeod's special tellurite medium and biochemical reactions have divided the strains isolated from these swabs as follows:

510 positive swabs.			Aberrant forms—starch and glycogen fermenters, but producing uniform turbidity in broth and no pellicle
<i>C. diphtheriae</i> <i>gravis</i> 16 (3.1 %)	Intermediate type 302 (59.2 %)	<i>C. diphtheriae</i> <i>mitis</i> 186 (36.5 %)	6 (1.17 %)

The "intermediate" type predominates and *gravis* forms are very rare. The aberrant forms correspond with two strains noted by McLeod in 47 Glasgow strains examined in his laboratory in 1932. The six strains above noted are the only variants from the three clear-cut types in this series. In colony appearance and growth in broth they resemble the *mitis* variety but they ferment starch and glycogen.

These results afford no support for the lack of correlation between colonial appearances and biochemical reactions described by Menton, Cooper, Duke and Fussell (1933), nor for the anomalies described by Wright and Rankin (1932). None of the difficulties of the latter authors with starch and cane sugar was experienced.

Tables I and II indicate the relationship between the type of bacillus and such clinical correlation as it has been possible to make. For comparative purposes they are drawn up on the same lines as the tables in the published papers of Anderson, McLeod and colleagues.

Table I. *Relation of type of bacillus in 271 cases of diphtheria.*

	<i>C. diphtheriae</i> <i>gravis</i>	Intermediate type	<i>C. diphtheriae</i> <i>mitis</i>	Aberrant forms
Rhinitis or carriers	1	16	18	—
Mild cases	5	18	27	2
Moderately severe cases	4	19	8	—
Severe cases	1	17	11	—
Haemorrhagic cases	—	3	—	—
Total	11	73	64	2
Albuminuria	1	28	10	—
Paralysis	1	10	7	—
Death	—	8	5	—
B. In 121 cases where end results only were available.				
Recovered	2	81	33	1
Died	—	3	1	—

Table II. *Percentage incidence of complications and deaths in 115 cases where detailed information was available (rhinitis and carriers excluded).*

	<i>C. diphtheriae</i> <i>gravis</i>	Intermediate type	<i>C. diphtheriae</i> <i>mitis</i>	Aberrant forms
Albuminuria	1	49.1	21.7	—
Paralysis	1	17.5	15.2	—
Death	—	14	10.8	—
No. of cases	10	57	46	2

*Percentage of deaths in 236 cases where end-results were available (rhinitis and carriers excluded).*

Deaths	—	7.8	7.5	—
No. of cases	12	141	80	3

The conclusions to be drawn from these figures are:

(1) That the "intermediate" type has been responsible for the severer cases of diphtheria in the Glasgow area during the past year. The *mitis* variety has run closely with the "intermediate," and the *gravis* type, which has been rare, has given rise to mild infections.

(2) That in infections with the "intermediate" type, paralysis and albuminuria are relatively common, which agrees with the findings of the Leeds School. In infection with the *mitis* type albuminuria occurs somewhat less frequently than the average. (Goodall (1928) states that albuminuria seldom occurs in less than 25 per cent. of cases of diphtheria.)

The number of cases of *gravis* infections in an area where diphtheria is on the whole mild (case mortality Glasgow, 1932, 5.52 per cent.) is too small to warrant any conclusion being drawn as to the virulence of the *gravis* type of organism. McLeod gives a morbidity rate of 13.1 per cent. in a study of 380 cases, with an incidence of albuminuria of 52.6 per cent. and of paralysis of 20.5 per cent.

#### VIRULENCE TESTS ON THE GUINEA-PIG.

These were carried out by the intracutaneous method (see Table III) exactly as described by Anderson, Happold, McLeod and Thomson, and the results therefore are comparable with those given in their paper of 1931.

Table III.

	<i>C. diphtheriae</i> <i>gravis</i> (14 strains)	Intermediate type (79 strains)	<i>C. diphtheriae</i> <i>mitis</i> (78 strains)	Aberrant forms (6 strains)
Very marked reactions	—	2	9	—
Marked	2	42	26	—
Moderate	5	17	15	2
Slight	7	12	11	4
Doubtful	—	1	2	—
Negative	—	5	15	—

The "intermediate" type shows more virulence for the guinea-pig, which is in accordance with its effect on man shown in the table of clinical correlations, in this series.

#### TYPE CHARACTERISTICS AND CONSTANCY OF TYPES.

Wright and Rankin (1932) found constancy of cultural characteristics in broth variable. Menton (1932, 1933) achieved similar results as did Parish, Whatley and O'Brien (1932). Variations in type characteristics have also been alleged. A large number of the cultures of *C. diphtheriae* dealt with in this paper were submitted to repeated sub-culture on various media, solid and liquid. They have been grown in serum broth made with antitoxic serum: have been frozen and thawed: heated short of death-point: have been recovered post-mortem after passage through guinea-pigs: have been left at room temperature exposed to diffuse light on a drying medium for several weeks: have been isolated from Loeffler's serum when almost overgrown by *B. subtilis*: have been grown under anaerobic conditions, and they have all been found to reproduce their original characteristics in colony form on tellurite medium, and in fermentation of carbohydrates, the only change noted being the development of very thin pellicles by some *mitis* strains in broth, but these pellicles were never comparable with the heavy surface growths over a clear fluid characteristic of *gravis* strains. The characteristic barred bacillary form of the "intermediate" type on tellurite was also preserved. The only variations in type characters were in the six aberrant strains before mentioned.

In one case in the series both *mitis* and "intermediate" forms were cultivated from a throat swab taken on the third day of the disease. This was a hospital case. Both varieties of organism bred true on repeated sub-culture. Both proved virulent in guinea-pigs (subcutaneous inoculation), the animals dying within 48 hours. The observations of McLeod and colleagues on such appearance of colonies of more than one type in primary culture have hitherto been negative. A single case like this proves nothing: the proximity of other cases in a diphtheria ward may be responsible for such a happening. This case recovered after treatment with 16,000 units of serum. The response was good, and except for slight albuminuria there were no complications.

## BIOCHEMICAL REACTIONS.

McLeod and his colleagues originally described the *mitis* type of *C. diphtheriae* as fermenting *d*-glucosamine. Noble and Knacke (1928) found that the Park Williams 8 strain was the only diphtheria strain which fermented it. Menton (1932) found that *d*-glucosamine failed to change in 26 cases where it should have done. In this series 30 *mitis* strains, 34 "intermediate" strains and 8 *gravis* strains were tested. Two samples of *d*-glucosamine were used, one obtained locally and one kindly supplied by Dr J. Gordon of Leeds. Using the method of Noble and Knacke, no definite evidence of fermentation was obtained in any case. A strain of P.W. 8 and 9 of the strains isolated by Eagleton and Baxter (1923), supplied from the National Type Collection, also failed to change *d*-glucosamine.

Haemolytic properties have been ascribed to *mitis* strains, but some observers have recorded anomalies. Tests have been carried out by Hammer-schmidt's (1924) method, using 12 *gravis* strains, 146 "intermediate," 142 *mitis* and 8 aberrant strains. 83 of the *mitis* strains displayed some slight haemolytic action, all others gave negative results.

## NOTE ON THE SPECIAL TELLURITE MEDIUM.

Bearing on the discrepancies noted by other authors in their analysis of diphtheria types, it is perhaps worth mentioning that in this study it has been found to be essential that the special tellurite medium of McLeod (1) be freshly made, (2) should contain not less than 5 per cent. blood, (3) should be adjusted to the optimum *pH*, 7.6, and (4) should not be overheated. The use of a medium diverging from these criteria gives rise to colonial formations which are misleading and may lead to errors in recognition of types. A year's experience with this medium provokes the opinion that for the isolation of *C. diphtheriae* from mixed cultures, in doubtful cases, and in cases where the bacilli are few, it is invaluable. It solves problems and saves time.

## GENERAL CONCLUSIONS.

1. In a study of 510 strains of *Corynebacterium diphtheriae* it has been possible to divide 99 per cent. into three types by colony appearance, the fermentation of starch, and appearance in broth, as described by the Leeds workers.

2. That in an area where diphtheria is mild, *C. diphtheriae gravis* is rare, and its place is largely taken by the "intermediate" type, which however is less virulent but causes paralysis and albuminuria to about the same extent as *gravis* when the latter is the chief infecting type.

3. The different types of *C. diphtheriae* have defined characteristics and are stable in these characteristics.

4. That diphtheria due to the "intermediate" type responds to antitoxic

serum in an average way, as with a large preponderance of cases due to this type, the case mortality rate is much below the rate in areas where the *gravis* type predominates.

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