

## THE DIFFERENT TYPES OF *CORYNEBACTERIUM DIPHTHERIAE*.

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IN 1890 Klein described two types of colony of the diphtheria bacillus on gelatin; one smooth and circular, the other radiating and resembling a daisy. Cowan (1927) observed two types of colony, but one of the types was thought to be due to roughening. Parker (1928) observed three types of colony, some of which were considered ordinary rough variants.

Hammerschmidt (1924) described two main types of *C. diphtheriae*, one showing a staphylococcus-like colony, turbidity in broth, and haemolysis, the other showing a flat ground-glass colony with spreading margin, a granular deposit in broth and no haemolysis. Dimitrejevic-Speth and Jovanovic (1931) suggested some correlation between the clinical manifestations and the mode of growth of the organism in agar gelatin and in a special broth.

Anderson, Happold, McLeod and Thomson (1931) brought forward evidence that *C. diphtheriae* could be classified into three types—Gravis, Mitis and Intermediate. They claimed that these different types could be distinguished by the characteristic colonial appearances when grown on a special chocolate tellurite medium. These types also showed other differences: Gravis produced a pellicle, early alkaline reaction and a granular deposit in broth, was non-haemolytic and fermented dextrin, starch and glycogen; Mitis caused turbidity and a later alkaline reaction in broth, was haemolytic, and did not ferment starch or glycogen; Intermediate produced a granular growth in broth and did not ferment the polysaccharides. They found that in Leeds Gravis types were correlated with the more severe cases and Mitis with the milder cases. They considered that these different types might have a bearing on serum treatment and that Gravis might be responsible for those severe epidemics which proved intractable to antitoxin. These different types were found to be stable.

Menton (1932) issued a preliminary report on the types prevalent in Staffordshire. The type of colony did not always agree with the other reactions. Moreover, several Mitis strains proved to be as virulent to guinea-pigs as Gravis strains, and Mitis was found to be responsible for severe clinical cases in man. Complement fixation was suggested as a possible means of differentiation. The terms "Gravis" and "Mitis" were deemed unsuitable on broad biological grounds. Strains showing Gravis colonies could be changed to Mitis *in vitro*

and some cases showed more than one type. No typical Intermediate types were observed during this preliminary investigation.

Parish, Whatley and O'Brien (1932), in a communication made at the same time, found that the mortality of rabbits to Mitis was higher than to Gravis. They found that the human death-rate for both types was approximately the same in different areas of England and Europe, and consequently concluded that the term "Gravis" is unjustifiable and that the Leeds findings appear to be a local phenomenon. These writers reiterated their views in a further publication (1932 *a*). They found that Park 8 antitoxin saved guinea-pigs inoculated with both types with equal readiness and prophylactic mixtures prepared from Park 8 and in current use for human immunisation were effective in protecting guinea-pigs against large doses of Gravis culture.

Table I.

Type of colony		Other reactions	Correlation
<i>(a) Cases and convalescents.</i>			
Gravis	70	Gravis throughout	Complete
Mitis	201	Mitis throughout	Complete
Mitis	11	Gravis throughout	Reversed
Gravis	21	Mitis throughout	Reversed
Gravis	37	Irregular	Partial
Mitis	56	Irregular	Partial
<i>(b) Carriers.</i>			
Gravis	9	Gravis throughout	Complete
Mitis	27	Mitis throughout	Complete
Mitis	2	Gravis throughout	Reversed
Gravis	1	Mitis throughout	Reversed
Gravis	6	Irregular	Partial
Mitis	4	Irregular	Partial

Anderson, Cooper, Happold and McLeod (1933), in a recent reply to these criticisms, gave their experience of an additional 500 cases. Their observations confirmed their previous experiences for the most part, but they found that Mitis may be more pathogenic to the guinea-pig than Gravis. In contradiction to O'Brien and others, they found Gravis more pathogenic to rabbits. They concluded with the statement, "The suggestion that Leeds Mitis strains are specially mild and that strains of this type will be found in other places equal in virulence for man with Gravis strains is probably true, but little evidence has till now been brought in support of it."

Our present communication is the outcome of an investigation conducted here during last year, during which cultures from 467 positive swabs were tested from cases, convalescents, and carriers.

In Table I the Gravis and Mitis strains from (*a*) cases and convalescents (396) and (*b*) carriers (49) are classified in respect to agreement and disagreement between the characters of the colonies produced on the Leeds chocolate-tellurite medium and the biochemical reactions described by the Leeds observers, as appertaining to the Gravis and Mitis types respectively.

It will be observed that in no less than 31.6 per cent. of the strains from

cases and convalescents the biochemical reactions were at variance to some degree with the colony character, *i.e.* a Mitis colony gave one or more of the Gravis reactions (pellicle and early alkalinity in broth, absence of haemolysis and fermentation of polysaccharides) and *vice versa*. With the strains from carriers the percentage of similar disagreement was 26.5. In 8 per cent. of the first set and 6 per cent. of the second the biochemical reactions were exactly the reverse of expectation from colony character: the disagreement among the others varied from one difference only, *e.g.* early alkalinity with a Mitis colony but otherwise Gravis in biochemical reactions, to five differences, *e.g.* general turbidity in broth, haemolysis and failure to ferment each of the three polysaccharides with a Gravis colony. No less than 25 different combinations of biochemical behaviour and colony characters appeared among these strains showing partial disagreement.

On the whole, the Mitis colonies agreed better with the expected biochemical behaviour (76 per cent. complete agreement) than the Gravis (55 per cent. complete agreement). Of strains with the Intermediate type of colony, as described by the Leeds workers, we had 20 from cases and convalescents and 2 from carriers. About half of these produced late alkalinity in broth; about a third produced a pellicle, a third of the latter producing late alkalinity and two-thirds early. Two of the 22 produced haemolysis and 4 fermented all three polysaccharides, the other 18 being polysaccharide-negative, except for dextrin fermented by 11 of them.

Our conclusion from all these observations is that we cannot rely on the appearance of the colonies as an index of biological type in the species *C. diphtheriae*. Since so many differences in observable biochemical behaviour occur among strains possessing the same sort of colony, it is reasonable to doubt whether the pathogenicity, *i.e.* the capacity to produce grave or mild disease in man, is likely to be more closely associated with colony appearance: and the same applies to any other character so far studied, *e.g.* polysaccharide fermentation, since, again, strains behaving alike in this capacity differ from each other in other respects. In other words, the bacterial character on which the known variation in severity of diphtheria in man depends has not yet been identified.

We have nevertheless attempted to correlate the severity of the clinical state with the different "types" in 185 cases. Information was supplied by the medical practitioners who had charge of the cases, and as several different people were concerned in assessing this severity it is doubtful whether the standards are quite comparable. Of the total 185 thus investigated, 114 were due to Mitis, 52 to Gravis, and 19 to Intermediate. Of the 114 Mitis, 96 were mild and 18 severe, giving a percentage severity of 15.8; in the 52 Gravis infections 37 were mild and 15 severe, giving 28.8 per cent. of severe disease; the Intermediates showed 15 mild and 4 severe, giving 21 per cent. severity.

The deaths are not included in the above figures. They were 4; 2 were due to Gravis. In one of these antitoxin was not given early, and the other patient

died after tracheotomy. This patient was very ill when the practitioner was called in and had obviously been so for some time before antitoxin was administered. Of the remaining two, one was due to typical Mitis; antitoxin was not given until comparatively late; and the remaining one was due to an atypical strain, but more closely resembling Mitis than anything else. In the first subcultures this strain showed large and small colonies which were non-haemolytic on first isolation but became haemolytic later and remained haemolytic in repeated subculture. This type at times fermented dextrin, and on one occasion gave off a type which fermented all the polysaccharides, and these reactions persisted. The colonies throughout were marked “? Mitis” for they were not absolutely typical. All subcultures of this peculiar strain gave turbidity and an early alkaline reaction in broth. This organism might be classified as an Intermediate in a state of instability, but in no way corresponding to the Intermediate types, as described by the Leeds workers.

In patients from whom swabs were submitted the number of deaths is too small to give any information on the main issue. We tried another line of approach. All the types recovered from carriers and cases were plotted out on a map of the county. The prevalent types in the different districts were compared with the total number of cases notified and the deaths occurring in these areas in 1932. In one area in the south of the county there were five deaths in a total of 30 cases. The swabs examined for this area showed 18 Mitis to 7 Gravis. In another area in the north of the county there was one death in 65 cases. The swabs from this area during the period April 1932 to March 1933 showed 15 Mitis, 20 Gravis, and 24 Intermediate. In still another area there were three deaths in 62 cases and the swabs received revealed 64 Mitis, 20 Gravis and 3 Intermediate. It is possible that, as we did not receive swabs from the rapidly fatal cases, all these may have been due to Gravis, but it is difficult to explain why, with so many Gravis strains about, the disease is showing a steady decline in mortality, from 15 per cent. in 1916 to 5 per cent. in 1931. A further point is that the Urban mortality as compared with incidence was only one-third of the Rural, which points to the ease of obtaining proper treatment as the main deciding factor; but still, there are other arguments.

#### OTHER OBSERVATIONS.

##### (1) *Persistence of type in the patient.*

Repeated swabs were examined from 84 patients. In 23, 6 at home and 17 in hospital, typical Mitis was obtained in every instance; and from 8, 1 at home and 7 in hospital, typical Gravis persisted. One otherwise typical Mitis strain fermented dextrin on every occasion.

Four patients showed strains on their tonsils which retained their Mitis characteristics except for fluctuations in their reactions on dextrin. In another case, two tonsillar swabs showed typical Mitis, but a third specimen taken from the same patient in hospital revealed Mitis colonies, an early alkaline

reaction in broth and fermentation of dextrin. A strain with indefinite colonies showed Mitis reactions, but on repeated swabbing, fermentation of dextrin and a pellicle and early alkaline reaction in broth occurred together from time to time.

A strain from a throat swab gave Gravis colonies and Mitis reactions. On repeating the swab, a strain with Mitis colonies and Mitis reactions, except for fermentation of dextrin, was obtained. Two Intermediate strains were recovered from the same patient at the same time; that from the throat fermented dextrin, but that from the nose failed to do so.

A nose swab from a hospital case yielded at first a typical Mitis and later a typical Gravis. Repeated throat swabs from a hospital patient showed a similar complete change from Mitis to Gravis. Several Mitis nose and throat swabs from hospital patients were at times non-haemolytic. One patient nursed at home showed Gravis three times and Mitis once. Variations in the broth reactions were also observed in repeated swabs. Two strains from hospital patients repeatedly showed Mitis colonies and Gravis reactions with a persistence which reassured us that such apparent anomalies were not haphazard nor due to errors in technique.

Different types from the nose and the throat were obtained in six instances. Four had Gravis in the throat and Mitis in the nose; two of these patients were in hospital. Two nursed at home had Mitis in the throat and Gravis in the nose. Two patients yielded strains with Mitis colonies, but the nose swab of one showed Mitis reactions and the throat swab Gravis reactions; the nose swab of the other showed Gravis reactions but was haemolytic, whereas the throat swab showed Mitis reactions. Gravis colonies were obtained from the nose and Mitis colonies from the throat of another patient, but both gave typical Mitis reactions.

The differences recorded above in strains recovered from the same patients were carefully checked, and when an expected biochemical reaction failed, precautions were taken to show that the micro-organisms were living and had grown freely in the tubes concerned.

#### (2) *Animal experiments.*

We have tested the virulence of 321 of our strains for the guinea-pig with the results shown in Table II. Each animal of approximately standard weight received intraperitoneally 1 c.c. (1800 million) of a broth suspension.

It will be seen that 74·3 per cent. of the virulent Mitis strains, 84 per cent. of the virulent Gravis strains and 87·5 per cent. of the virulent Intermediate strains killed the animals within 48 hours.

We have found from the following experiments that commercial antitoxin provides protection against Gravis and Mitis strains and that this protection is not selective.

Two groups of four guinea-pigs were taken, each weighing 250 g. One group received 1 c.c. (1800 million) Gravis culture and the other 1 c.c. (1800

million) Mitis culture intraperitoneally. The cavies were given 250 units antitoxin intraperitoneally 1, 3, 6 and 23 hours later. All the animals were alive and well 10 days later. Controls with Mitis and Gravis cultures were dead on the fifth and second day respectively.

In spite of the greater virulence of the Gravis strain, complete protection was afforded.

Table II.

Day of death	Cases			Carriers		
	Mitis	Gravis	Inter- mediate	Mitis	Gravis	Inter- mediate
1st day	46	20	9	12	5	—
2nd „	44	17	5	5	—	—
3rd „	19	2	—	—	1	—
4th „	4	3	—	2	—	1
5th „	6	1	—	1	—	—
6th „	2	1	2	1	—	—
Negative	73	18	3	11	6	1
Total	194	62	19	32	12	2

CONCLUSIONS.

A. The strains of *Corynebacterium diphtheriae* in Staffordshire cannot be classified into three definite types on the colonial appearances plus the biochemical reactions as described by the Leeds workers.

B. The lack of correlation between the appearances of the colonies and the biochemical reactions is sufficiently pronounced to throw doubt on the type specificity of the colonial characters.

C. The fermentation of polysaccharides may afford a better index for classification.

D. We have been unable to justify the term "Gravis" in its original sense.

E. We have not found any type selectivity with antitoxin.

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N.B. The medical contributors (J. M. and T. V. C.) are solely responsible for the medical opinions and animal experiments in this investigation.

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