BIOLOGICAL TYPES OF THE DIPHTHERIA BACILLUS

AND THEIR CLINICAL CORRELATION.

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COPY.

Dr. A. C. Morrison has asked for my permission to incorporate for the purposes of an M.D. thesis certain bacteriological results which I have obtained in the course of work in collaboration with Dr. Leete and himself. I give this permission most willingly.

Actually a considerable number of these bacteriological investigations have been duplicated by the workers at Cottingham and with one minor divergence the findings have been identical.

(Signed) J. W. McLeod.

Professor of Bacteriology,

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29th March, 1933.

PREFACE.

"In view of the unusual severity of cases of diphtheria admitted to the City Fever Hospital, Hull, during the past year it was deemed only proper to take opportunity to confirm the findings of the workers at Leeds, whose claim to have established a correlation between a virulent form of the bacillus diphtheriæ, described as 'gravis', and its clinical manifestations is one of no mean importance.

The following thesis presents the results of such a confirmatory investigation.

The writer's thanks are due to Professor J. W. McLeod, of the Chair of Bacteriology in the University of Leeds, for permission to publish certain findings, and also to Dr. H. Mason Leete, Superintendent of the City Fever Hospital, Hull, who kindly granted the necessary facilities for the enquiry."

--ooOoo--

INTRODUCTION.

The aim of this thesis is to determine the relationship, if any, between the severity of cases of diphtheria and the type of infecting organism. All the cases investigated have occurred within the City of Hull.

In the later months of 1931, certain disquieting features were noticed with regard to the cases of clinical diphtheria admitted to the City Fever Hospital, Hull.

It was noted that there was a considerable rise in the number of these cases. While the maximum prevalence of the disease usually obtains in the last quarter of the year, yet the incidence was out of all proportion to anything attributable to seasonal influences.

The general impression received was that coupled with the rise in numbers there was an increase in severity of infection and of all admissions, a much greater percentage could be classified as suffering from a severe attack of diphtheria.

A third disturbing feature which demanded recognition, was the poor response in many cases to what appeared to be adequate treatment with antitoxin.

It is very instructive to study the following table compiled from the records of the hospital for the past seven years, during which period the population of Hull has increased only 0.06 per cent. (1) :-

TABLE 1.

Cases admitted to Hull City Fever Hospital as Diphtheria 1926-1932 with Mortality Figures.

Year	Cases admitted as Diphtheria.	Deaths from Diphtheria.
1926	682	18
1927	721	33
1928	635	21
1929	826	30
1930	828	43
1931	1154	98
1932	1706	123

The figures referring to 'cases admitted as diphtheria' require correction for errors in diagnosis and for cases admitted as carriers, but, despite this fact, these figures are a fair indication of the incidence of the disease. The

mortality figures are particularly significant, and are probably a more reliable index of severity than other statistical data. This is because they represent, of necessity, severe diphtheria; and as all the deaths enumerated occurred in hospital, the diagnosis in such cases may be regarded as having been firmly established.

All these factors point to some alteration either in the resistance of the community as a whole, or in the type of infection, and it will be possible to show that the latter is the dominating influence.

In 1931 Anderson, Happold, McLeod and Thomson (2) published results of an investigation on diphtheria in Leeds in which they suggested a correlation between different forms of the diphtheria bacillus, as described by them, and the clinical features of the cases with which these forms were associated. In view of the experiences already related, it seemed appropriate that a similar investigation might be undertaken here.

It is the aim of the thesis to show that the types of diphtheria bacillus described by the Leeds workers have both existence and clinical correlation, in this area.

HISTORICAL OUTLINE.

The history of diphtheria dates from ancient times. It would appear that it was first observed clinically by Hippocrates who says, according to Currie, (3) that in tonsillar ulceration a webby exudate is 'no good thing'.

According to the translation by Adams (4) in the Third Book of Epidemics, in the Corpus Hippocraticum, an account is given of a case in which the following features were present :- inarticulate character of speech, reddened hard swellings on both sides of the neck, coldness and lividity of extremities, and regurgitation of fluids by the nose.

While this fits in admirably with the clinical picture of modern diphtheria, it is possible that it does not refer to this disease, but there is almost universal agreement among medical historians that there were described in the second century, careful clinical observations clearly relating to diphtheria. Again, in the sixth century, Aetius describes ulcers on the tonsils which were followed, as patients began to recover, by thick speech and by the return of fluids by the nose.

As Currie (5) points out there was, in Rome, in the year 1004, an outbreak of diphtheria attended by high mortality.

Little else of note appears in the records until the sixteenth and seventeenth centuries, when in Europe, and particularly in Spain, there were severe epidemics, and a study of minute accounts of these shows that they can be attributed to diphtheria. Indeed, it does not seem possible that these accounts can refer to any disease other than diphtheria.

In Britain perhaps the first recognition of diphtheria was in the early part of the eighteenth century when a case of 'putrid sore throat' is said to have been identified as the severe disease which had been prevalent in Spain. Francis Home of Edinburgh, in 1765, published a dissertation on 'croup' and his views soon became widely accepted. He was doubtless writing about diphtheria.

The clinical manifestations of diphtheria continued to be recognised during the nineteenth century, but the next important development was in 1883 when Klebs discovered the causal organism. In the following year,

this organism was recovered by Löffler from cases of clinical diphtheria. In tribute to these workers the organism received the name which it still bears, that of the Klebs- Löffler bacillus. Roux and Yersin in 1888-90 published results of experiments which established definitely the relationship of the Klebs-Löffler bacillus to diphtheria.

With the epoch-making discovery of antitoxin by von Behring in 1890 and following its remarkable therapeutic success, it appeared that an efficient method had been evolved for the treatment and control of diphtheria, and certainly for a long period, results seemed to justify this view.

During the past few years, however, a steady increase in the mortality rate of diphtheria has been noted in many quarters. This more severe type of diphtheria was first observed in Germany in 1927, and more recently, epidemics of unwonted severity have been reported from all parts of Europe. In 1929 Potter (6) recorded cases of malignant diphtheria in America, and Hercus and Wilson (7) state that in New Zealand during the years 1929-1931 an increased case mortality coincided with

the appearance of an exceptionally malignant type of the disease.

While in this country, during 1931, decline in diphtheria prevalence was the rule, the disease has, in certain areas, presented formidable features. According to a survey of diphtheria by Forbes (8) Hull is such an area.

CLINICAL CLASSIFICATION.

Since diphtheria is most commonly faucial, this variety may conveniently be regarded as a type and all cases of clinical diphtheria dealt with in this investigation were of the faucial type. Cases of laryngeal diphtheria were also investigated but the number of these was relatively so small, that it was not considered that any benefit would accrue from their inclusion.

Each case in the series was seen within one or two hours of admission to hospital and was classified on clinical grounds at the first examination. It not infrequently happened that a case appeared much better or much worse the day after admission, but no modification was made in the light of subsequent progress, the original classification being adhered to in every case.

The features considered were :-

1. Foetor
2. Degree of adenitis
3. Extent of throat membrane
4. Surrounding inflammation and oedema of throat.
5. General clinical impression.

According to the presence or absence of these features in varying degree, the cases were divided into the following groups :-

1. Mild.
2. Moderate.
3. Severe.

With the increasing number of severe cases a further sub-division was made :-

Severe 1.
Severe 2.
Severe 3.

The type classified as Severe 3. is usually an example of rhino-faucial diphtheria presenting extensive faucial membrane with perhaps spread to the posterior nares. Adenitis is bilateral and profound and frequently attended by discolouration of the skin. The patient is markedly toxaemic and is obviously acutely ill. The prognosis in such cases is well nigh hopeless.

BACTERIOLOGICAL CLASSIFICATION.

Muir and Ritchie (9) and other standard textbooks stress polymorphism as a feature of the Klebs-Löffler bacillus and describe long, short, club-like and barred forms as well as branched forms. Almost invariably two or three of these forms are present in the same culture, although one commonly predominates. Peters (10) however, states that there are two morphological species of the pathogenic diphtheria bacillus, and that these do not show mutation. He states further, that on several occasions he has isolated cultures solely of short forms. While confirmed by other workers, such an occurrence is extremely rare.

Many attempts have been made to classify strains of the diphtheria bacillus on morphological bases. Wesbrook and others (11) carried out extensive work on these lines and finally allowed that every culture consisted of a mixture of practically all the forms of the bacillus.

Agglutination methods have been described by Bell (12) and Havens (13) but do not now seem to be generally acceptable

as a means of classification. In this direction, work has been severely hampered by difficulties of emulsification in the case of many organisms.

In 1931, Anderson, Happold, McLeod and Thomson (2) published an account of a classification of strains of the diphtheria bacillus on biological grounds. They classified the bacillus into three types - Gravis, Intermediate and Mitis. The essential features of each type are given:-

Gravis - grows with granular deposit, often coarse, and pellicle in broth, and actively ferments starch.

Intermediate - gives fine or medium granular growth in broth, and does not ferment starch.

Mitis - grows with uniform turbidity in broth and does not ferment starch.

The methods of these workers, with some modifications, have been adopted in arriving at the bacteriological classification in this investigation.

Of the total number of 220 cases investigated, the bacteriology in 105 has been worked out by Professor J. W. McLeod of The University of Leeds, and he has

kindly granted me permission to use his figures. In the remaining 115 cases, the bacteriological classification has been determined by the writer whose procedure has been as follows :-

From a swab taken from the throat, inoculation is made on a Löffler's serum slope. This is incubated for 12 hours at 37° C. and films are made from the resulting culture. These are strained by a modified Neisser method using Picroerythrosin as the counterstain, and are examined for bacilli which show the morphological characteristics of the diphtheria bacillus. It may be necessary to swab the throat on several occasions before a positive result is obtained.

A sample of the growth from the positive culture is emulsified in five cubic centimetres of normal saline, and a glucose-tellurite-calcium-malate-serum plate is inoculated with a loopful of the emulsion. The medium is a slight modification of that introduced by Conradi and Troch in 1912. Full details are subjoined :-

The glucose tellurite calcium malate serum is prepared as follows :-

Lab Lemco	10 grms.
Sod. chloride	5 grms.
Witte's peptone	20 grms.
Calcium Acid Malate ...	6 grms.
Glucose	10 grms.
Distilled water	1000 c.c.

Mix. Steam for 30 minutes. Filter hot. Stand overnight. Filter cold. Sterilise in Koch (30 minutes on three successive days).

Label glucose malate broth.

Take :-

Glucose malate broth	25 c.c.
Seitz filtered (E.K.film)	75 c.c.
Potassium tellurite 1% solution ..	2 c.c.

Mix well and pour plates. Inspissate for 1 hour at 80° C.

The resulting medium should be light in colour and printed matter should not be able to be read through it.

The plate is examined at the end of 48 hours incubation and colonies of all the types which suggest diphtheria bacilli are picked off, and inoculated on a Löffler's slope. Each tube is then incubated for 12 hours, after which films of the growth are made, stained and examined. It has been found that as a rule, it is a black colony of medium size, either uniform 'solid black' or with a hazy border, which proves to be a morphological diphtheria bacillus. Streptococci show as minute black colonies. Very large black colonies usually turn out to be cocci, so that extremes of size may be neglected.

Having determined that a certain Löffler's serum slope is growing what appears to be a pure morphological diphtheria bacillus, this is regarded as a primary culture and

inoculation is made from it into the

following tubes :-

1. Peptone serum water - plain.
2. Peptone serum water plus 0.5 per cent Starch.
3. Peptone serum water plus 1 per cent Glucose.
4. Peptone serum water plus 1 per cent Saccharose.
5. Peptone broth.

The peptone serum water is specially prepared by a modified technique of which details are now submitted :-

Peptone Serum Water

Difco Bacto-peptone	5 grms.
Acid sodium phosphate	1 grm.
Distilled water	1000 c.c.

Steam 15 minutes. Filter hot. Cool. Add 250 c.c. Seitz filtered (E.K.film) Ox Serum. Steam 30 minutes. Cool.

Add Bromothymol blue (0.2% alcoholic solution) 12.5 c.c.

Add sufficient N/1 NaOH. to bring the colour to a green tinge in which blue is just beginning to appear. About 7 c.c. is a usual figure. Actually N/10 NaOH is titrated against 10 c.c. of the medium, and the colour is brought to that of a sealed standard; subsequently adjusting bulk proportionately with N/1 NaOH. The final test corresponds to a pH value approximately of 7.2, which is alkaline.

Mix. Steam 30 minutes. Leave overnight and steam following day for 30 minutes. Label Peptone serum water.

Carbohydrates are added in the proportion of 1 per cent. for Glucose and Saccharose, and 0.5 per cent. for starch.

In the case of Glucose, addition is made from a sterile 10 per cent. stock solution.

Saccharose is sterilised dry in the hot air oven for 2 hours at 150° C. and then added. If kept in stock solution it often hydrolyses even at room temperature.

0.5 grms. of Starch (B.D.H. A.R. soluble) is similarly dry sterilised, mixed with 10 c.c. peptone-serum-water and to this is added 90 c.c. of peptone-serum-water at 100° C. and the whole is stirred vigorously so as to obtain a uniform suspension.

After addition of carbohydrates and distribution in tubes, a final sterilisation of 10 minutes in the Koch is given.

The peptone broth is a meat infusion broth with a pH of 7.4.

Each of these tubes is included in the series for a particular reason which may here be indicated.

1. Peptone serum water - plain - this tube is to show that there is no acid production from the medium, without added carbohydrate. It is kept as a stock culture for any further investigation which may be desirable.
2. Peptone serum water plus starch - this tube is a criterion for biological classification.
3. Peptone serum water plus Glucose - this is a control tube as all diphtheria bacilli ferment glucose.
4. Peptone serum water plus Saccharose - this is a further control, as no true diphtheria bacilli ferment saccharose.
5. Peptone broth - this tube provides readings for bacteriological classification into biological types.

All these tubes are incubated at 37° C. At the end of 24 hours broth readings are made, when the following features are studied :-

Pellicle formation, turbidity, deposit, and granularity or otherwise of the deposit on shaking. At the end of 48 hours incubation, the broth tubes are re-read as an additional precaution, and all other tubes are read for results of fermentation. The organism under examination can then be classified into its biological group of Gravis, Intermediate or Mitis.

TABLE 11.

Biochemical Reactions of Typical Diphtheria Strains.

	Peptone serum water -plain.	Peptone serum water plus Starch.	Peptone serum water plus Glucose	Peptone serum water plus Saccharose.	Peptone Broth.
Gravis	-	AC	AC	-	G
Intermediate	-	-	AC	-	G
Mitis	-	-	AC	-	T

- = no change takes place.

AC = acid and clot formation.

G = Granule formation.

T = Turbidity.

With the indicator used acid formation produces a yellow colour.

Clotting with both starch and glucose is quite firm in 48 hours.

THE CLINICAL CORRELATION OF BIOLOGICAL TYPES OF THE DIPHTHERIA BACILLUS.

This investigation consists of a study of 220 cases treated in all instances to a conclusion. The cases have not been in any way selected, but include all admitted to hospital within a definite period, in which the bacteriological diagnosis was established after admission. There has been obtained therefore, a true representation of hospital admissions, and so of the disease in this area, covering the period under consideration, since almost every case of diphtheria is hospital treated.

Virulence tests have been carried out in a high proportion - over 90 per cent of the cases; and all organisms tested were found to be virulent.

The biological type of the infecting bacillus was determined in all cases. In a certain number, however, there was no definite evidence of clinical disease, and such cases were classified as 'carriers' and will more satisfactorily be studied as a subsidiary part of the investigation.

These Carriers number twenty and they may conveniently be studied at this stage. They form only a small group, and are not discussed at length.

TABLE 111.

Biological Types of Diphtheria Bacillus in Carriers.

Type of Carrier.	Number of Carriers.	Biological type of diphtheria bacillus.		
		Gravis	Intermediate	Mitis
Faucial	7	3	2	2
Nasal	13	2	4	7

From a table compiled from such small numbers, the only conclusion to be drawn is that both faucial and nasal carriers may harbour any one biological type of the diphtheria bacillus.

BIOLOGICAL TYPES OF THE DIPHTHERIA BACILLUS IN 200 CASES OF CLINICAL DIPHTHERIA STUDIED.

TABLE IV.

The Sex Distribution of Biological Types of the Diphtheria Bacillus.

Sex	Number of Cases.	Percentage of Gravis cases.	Percentage of Intermediate cases.	Percentage of Mitis cases.
Males	91	61.5	28.6	9.9
Females	109	65.1	23.9	11.

As might be expected, sex does not play any part in determining the biological type of infection in clinical cases of diphtheria.

TABLE V.

The Age Distribution of Biological Types of the Diphtheria Bacillus.

Age Group	Number of cases in Group.	Percentage of Gravis Cases.	Percentage of Intermediate cases.	Percentage of Mitis cases.
Under 5yrs.	49	59.2	30.6	10.2
5 -	103	65	23.4	11.6
10 -	35	60	31.4	8.6
15 -	8	75	25	0
20-42 years	5	80	0	20

As shown in this table only the first three age groups are composed of sufficiently large numbers to justify of any conclusion being drawn. It appears that the biological types of diphtheria bacillus are distributed, more or less uniformly, irrespective of age.

In the 200 cases of clinical diphtheria under discussion, the biological types of the bacillus are grouped as follows :-

Gravis	... ...	63.5 per cent.
Intermediate	... ...	26 per cent.
Mitis	... ...	10.5 per cent.

There is, therefore, a high incidence of Gravis infection.

When clinically classified, a high proportion of these 200 cases is shown to be of a 'severe' type.

Severe	... ...	40.5 per cent.
Moderate	.. ..	31.5 per cent.
Mild	... ...	28 per cent.

TABLE VI.

Clinical Classification of the 200 Cases in Correlation with the Biological Type of Infection.

Biological type.	Number of Cases.	Percentage of Severe cases.	Percentage of Moderate cases.	Percentage of Mild cases.
Gravis	127	47.2	27.5	25.3
Intermediate	52	36.5	38.5	25
Mitis	21	9.5	38.1	52.4

The 'gravis' type of infection clearly shows the highest percentage of severe cases. Similarly the 'mitis' type of infection shows the highest percentage of mild cases.

It is of some importance to note that the 'intermediate' type is associated chiefly with a case of moderate degree of infection. This was not expected as the term 'intermediate' is used purely as a bacteriological classification. This table shows that it is also intermediate in clinical significance.

TABLE V11.

Biological Type of Infection in Correlation with Clinical Classification.

Clinical Classification.	Number of Cases.	Percentage of Gravis cases.	Percentage of Intermediate cases.	Percentage of Mitis cases.
Severe	81	74.1	23.4	2.5
Moderate	63	55.6	31.7	12.7
Mild	56	57.1	23.2	19.7

It is definitely indicated by the above table that the highest percentage of 'mitis' infection is found among mild, while the highest percentage of 'gravis' infection occurs in the severe cases. The moderate cases are intermediate in position and show a higher percentage of infection with the 'intermediate' type than mild or severe cases.

The Severe cases can be further subdivided :-

TABLE V111.

Biological Type of Bacillus in Severe Cases.

Clinical Classification.	Number of Cases.	Percentage of Gravis cases.	Percentage of Intermediate cases.	Percentage of Mitis cases.
Severe 1	41	61	34.1	4.9
Severe 2	37	86.5	13.5	0
Severe 3	3	100	0	0

The numbers in this table are small, but they indicate that with an increasing clinical severity there is a considerable rise in the percentage of 'gravis' infections, and a corresponding decline in 'mitis' and in 'intermediate' infections. The fall is most noted in the 'mitis' type of infection.

Relation of Biological Type of the Diphtheria Bacillus to Fatal Cases and to Paralytic Complications.

Of the 200 cases of clinical diphtheria, 20 per cent. were fatal and a further 12 per cent. developed one or more forms of paralytic complication during recovery. The former high figure is due to the unusually large number of fatal cases admitted towards the end of the investigation.

TABLE IX.

Deaths and Paralyses according to Biological Classification, rigidly controlled by Cultural and Virulence Tests.

	Number of Cases.	Percentage of Gravis Cases.	Percentage of Intermediate cases.	Percentage of Mitis Cases.
Deaths	40	87.5	12.5	0
Paralyses	24	75	20.8	4.2

The percentage of deaths and paralysis is high in 'gravis' infection. Throughout the investigation there has not been a fatal case where the mitis type of infecting organism has been concerned, and there has been only one case of paralytic complication on mitis infection.

DISCUSSION.

Serum and other therapy.

The treatment of all the cases of clinical diphtheria which have been studied, has been simply that carried out as routine in the City Fever Hospital, Hull. Essentially it has consisted of administration of anti-diphtheritic serum and rest in bed.

Serum is given as soon as possible after admission to hospital. Where the case is at all severe, part of the total amount of serum - usually about one-third of what is considered an adequate dose - is given by the intravenous route. Doses of antitoxic serum ranging from 4000 units for a mild case to 60,000 units, or in some instances 80,000 units, for a severe case, have been given. The adoption of 60,000 units as the ordinary maximum dose follows upon the results obtained in a series of severe cases in which amounts of serum up to 200,000 units were given. In this series of cases, massive doses of serum did not appear to produce results of a more satisfactory nature.

Apart from serum therapy, treatment has been directed mainly on two lines. In most patients suffering from a moderate or severe attack of diphtheria, some form of tonic treatment is generally employed. Strychnine, pushed when necessary, is found to be the most satisfactory tonic. Mainly its use is to endeavour to lessen the severity of paralytic complications.

In the treatment of complications, strychnine, adrenalin, and in suitable cases, morphine, have all been employed but, with the possible exception of strychnine, their value in serious complications is doubtful. Atropine is occasionally beneficial as a depressant of the salivation mechanism.

Observations on the writer's findings.

The number of cases in this investigation is admittedly not large, but it seems fairly clear from the data given that there is a distinct correlation between the clinical type of diphtheria and the biological characteristics of the associated organisms.

In 200 cases of clinical diphtheria there are 127 showing infection with the 'gravis' type of diphtheria bacillus, and a large proportion of these, namely 47.2 per cent. is classified as clinically severe. Furthermore, as the cases increase in clinical severity, so does the percentage of 'gravis' infection rise. 'Gravis' infection accounts for 87.5 per cent. of the fatalities and 75 per cent. of the paralytic complications in the cases under review.

The 'intermediate' type of diphtheria bacillus was isolated in 52 instances. Of the fatal cases, 12.5 per cent. come into this biological classification, as do 20.8 per cent. of the cases showing paralytic complications.

There are only 21 examples of the 'mitis' type of infection in the 200 cases of clinical diphtheria. A very small proportion of these, namely 9.5 per cent. were classified as clinically severe. There has not been a fatal case from 'mitis' infection and only one of this group showed a paralytic complication.

Findings of other workers.

Having presented the results of this investigation in some detail, reference may be made to the results of similar work by others.

In 1931 Anderson, Happold, McLeod and Thomson (2) published their biological classification of types of the diphtheria bacillus, and showed the relationship of the variety of the bacillus to the clinical condition in 104 cases. These workers, at this stage, described two principal forms of the bacillus, the B. diphtheriæ 'gravis' responsible for severe cases of the disease, and the B. diphtheræ 'mitis' associated with milder cases. They stated also that 'intermediate' forms possessing certain features of 'gravis' and certain features of 'mitis' types had been observed in a small percentage of cases.

Wright and Rankin (14) in 1932, reported the study of a series of strains of diphtheria bacilli isolated from different cases in Edinburgh, and correlated with the clinical records of the cases. They were

unable, however, to establish any correlation of clinical condition with biological type of bacillus. They suggested that 'Possibly the true 'gravis' form does not occur in Edinburgh at present' and this would appear to be the case, as their paper records no definitely starch-fermenting strains. It is significant that in Edinburgh, diphtheria has been relatively a mild disease in recent times.

Parish, Whatley and O'Brien (15) give figures which indicate that infection with the 'gravis' type of organism occurs in many areas. They do not find any satisfactory evidence that severe cases of diphtheria are usually associated with the 'gravis' type of bacillus. These workers do not, however, give any detailed analysis of those cases exhibiting the 'intermediate' type of infection which they appear to disregard. But they have proved that in guinea pigs an equally satisfactory response follows the use of ordinary anti-diphtheritic serum whether the infection be of the 'gravis' or 'mitis' type.

With reference to the experimental work on animals of Parish, Whatley and O'Brien it has been shown by Anderson, Cooper, Happold and McLeod (16) that very mild or severe infections in man, may not reproduce their features in the experimental animal.

CONCLUSIONS.

1. Biological types of the diphtheria bacillus, as described by the Leeds workers, are active in Hull, and can easily be classified by a simple technique which the writer has described.

2. The biological types have a definite clinical correlation in Hull.

3. The incidence of 'gravis' type of infection in Hull is high.

4. A large proportion of severe cases are of the 'gravis' type.

5. A still higher proportion of fatal cases yield the 'gravis' type of bacillus.

6. The greater percentage of 'mitis' type cases are clinically mild.

7. The 'intermediate' type of diphtheria bacillus occupies a place intermediate in clinical significance.

REFERENCES.

1. Annual Reports of the Medical Officer of Health of Hull. 1926-32.

2. Anderson J.S., Happold F.C., McLeod J.W., and Thomson J.G. Journ. Path. & Bact. 1931. XXXIV. p.591.

3. Currie J.R., "A Textbook of Hygiene". Livingstone. 1930. p. 400.

4. Adams F., Trans. "The Works of Hippocrates" 1849. I. p.p. 394, 395.

5. Currie J.R., "A Textbook of Hygiene". Livingstone. 1930. p.401.

6. Potter P.S., Archives of Pediatrics. 1929. XLVI. p.p. 431-436.

7. Hercus C.E., and Wilson H., Brit. Med. Journ. 1932. p.p. 873, 874.

8. Forbes J.G., Journ. State Med. 1933. XLI. No. 3. p. 148.

9. Muir R., and Ritchie J., "Manual of Bacteriology". Oxfd. Med. Pub. 1927. p.p. 463, 464.

10. Peters E.A., Journ. Path & Bact., 1897. IV. p.p. 188, 191.

11. Wesbrook F.F., Wilson L.B., McDaniel O., and Adair J.H., Brit. Med. Journ. 1898. p.p. 1008, 1009.

12. Bell A.S.G., Journ. Royal Army Med. Corps. 1922. XXXVIII. p.p. 48 - 53.

13. Havens L.C., Journ. Inf. Dis., 1920 XXVI. p.p. 393-395.

14. Wright H.A., and Rankin A.L.K., Lancet. 1932. p. 884.

15. Parish H.J., Whatley E.E., and O'Brien R.A., Brit. Med. Journ. 1932. p. 916.

16. Anderson J.S., Cooper K.E., Happold F.C., and McLeod J.W., Journ. Path & Bact. 1933. XXXVI. p. 169.