

A SURVEY OF THE BIOLOGICAL TYPES OF THE
DIPHTHERIA BACILLUS IN EDINBURGH,
1932-1939

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SINCE the introduction of diphtheria antitoxin nearly fifty years ago, diphtheria has been generally regarded as a disease which is readily amenable in its early stages to specific therapy. Moreover, in recent times, the disease as it occurs in Europe has rarely assumed the severe epidemic form recorded in its earlier history. Some degree of alarm was therefore caused some years ago when severe epidemics were reported from the Continent (Deicher and Agulnik, 1927), and later from England, (Anderson *et al.*, 1931) when the majority of the cases were of a highly toxic nature, often rapidly fatal and frequently exhibited no response to antitoxin treatment.

An epidemic of this kind began in Leeds in 1930 and led to a series of bacteriological studies which have created a somewhat new outlook on the ætiology of the disease. At that time, McLeod and his associates were able to define three different biological types of the diphtheria bacillus designated *B. diphtheriæ gravis*, *mitis*, and *intermedius* respectively (Anderson *et al.*, 1931), and these workers have regarded such types as distinct and stable. The *gravis* type was found to be associated at that time with the toxic form of the disease which was prevalent in the epidemic in Leeds, the *mitis* type was associated with the milder cases. Only a negligible proportion of cases were due to the *intermedius* form. The important clinical question then arose as to whether these types all produced the same or different toxins. It seemed possible that the disappointing therapeutic effects of diphtheria antitoxin in the severe cases might be due to a difference in the toxin of the *gravis* type and that of other strains. This question however has been finally settled (Parish, Whatley and O'Brien, 1932; Parish and Wright, 1935), and the complete identity of the toxins of the different types has been clearly established. The therapeutic failure referred to can only be attributed to the essential severity and rapidity of the illnesses dealt with at that

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time, and illustrates an important limitation of specific serum treatment.

Since 1931, the types of diphtheria bacillus and their epidemiological relationships have been most extensively studied in many different countries, and there is now a voluminous literature on the subject. Such investigations were initiated in Edinburgh in 1931; and, in order that a complete picture might be obtained of the part played by these organisms at different times and under different conditions, the study was extended until 1939. In 1932-33, 60 per cent. of cases of diphtheria which occurred in Edinburgh were found to be due to the *intermedius* type, 20 per cent. to *mitis*, and infections by the *gravis* type were almost completely absent—a finding entirely different from the state of affairs in Leeds. The Edinburgh district was, however, free from the virulent form of the disease which had been recorded in other areas. In the course of these studies in Edinburgh, reports of which were published in 1935 (Wright *et al.*, 1935) and 1936 (Christison, *et al.*, 1936), three other biological types of the diphtheria bacillus were described; and the conclusion was drawn that the various types lacked complete stability as regards the biological characters according to which they were defined. It is not proposed in this paper to deal with the purely bacteriological details of the investigation, but Table I summarises the differential characters of the six types referred to. These have been designated numerically, I to VI.

It may be noted that slight variation of colony form was found to be of frequent occurrence, and influenced to a great extent by small differences in the medium such as uneven drying and the length of time it had been stored, and possibly also by the inherent instability of the strains when newly isolated. In contradistinction to other workers who have found the results of hæmolytic tests equivocal (Robinson, 1934; Stuart, 1938), this test when carried out with ox and rabbit cells in parallel (Christison, 1934-5) was found of value especially in distinguishing Types I and III from Types II, IV and VI (see Table I). Prior to 1935, the few Type III strains which were isolated in Edinburgh did not lyse rabbit's erythrocytes (Wright, *et al.*, 1935); but since then only a small proportion of starch-fermenting strains failed to produce hæmolysis for rabbit red cells. Ox red cells were lysed by Type I only.

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Throughout the investigation the material was drawn from the Edinburgh area. Before 1935 the study was not continuous; but since then, except for short holiday periods when a few cultures were omitted, it has included all ear, nose, and throat cultures reported by the University Bacteriology Department to contain organisms with the morphological characters of *B. diphtheriae*. By the courtesy of Dr W. T. Benson, Medical Superintendent of the Edinburgh City Hospital for Infectious Diseases, and his successor, Dr Alexander Joe, these included cultures from all patients in the hospital suffering from diphtheria, *i.e.* the large majority

TABLE I
Characters of Biological Types of B. diphtheriae

Type.	Designation of Anderson <i>et al.</i>	Colony Characters on McLeod's tellurite-rabbit-blood-agar.	Fermentation of starch.	Hæmolysis.		Virulence to guinea-pig.
				Ox.	Rabbit.	
I	<i>mitis</i>	Convex, round, glistening	—	++	+++	+
II	<i>intermedius</i>	Small, lustreless, with domed centre and flat irregular margin	—	—	—	+
III	<i>gravis</i>	{ Lustreless, typically "daisy head," with raised centre and sloping, striated, irregular margin }	+	—	++	+
IV	...		—	—	—	+
V	...		+	—	—	—
VI	...		—	—	—	—

of cases notified in Edinburgh. Clinical notes and an assessment of the clinical severity of each case were supplied by the senior assistants in the hospital. A few cultures were also drawn from the Royal Hospital for Sick Children.

Since September 1936, each year has been arbitrarily divided into four periods: 1st January to 28th February, 1st March to 31st May, 1st June to 31st August, and 1st September to 31st December. It may be noted that the number of cases has diminished since the middle of 1938. This is a reflection of the fall in notifications of the disease in Edinburgh* and not the result of any change in the method of collecting the material. Details of the bacteriological findings in the different periods are set out in Table II, and the outstanding points of epidemiological interest are illustrated in the chart, p. 545.

* January to May 1938, 386; 1939, 184.

TABLE II

Incidence Per Cent. of Clinical Classes and Bacteriological Types

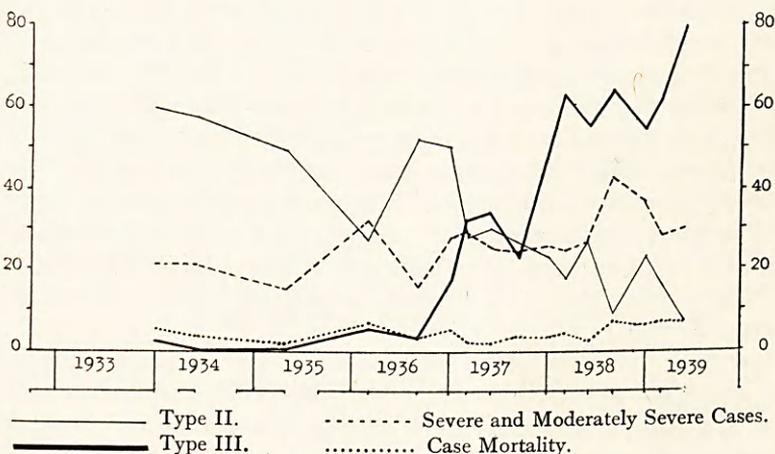
Date.	Severe.*	Moderately Severe.	Mild.	Carriers.	Fatal.	No. of Cases.	Biological Types.						
							I.	II.	III.	IV.	VI.	Atypical.	Cases due to Two or more Types.
1936 March.-Aug. Sept.-Dec.	8.3	7.5	46.7	37.5	3.3	120	23.4	52.5	3.3	10.0	5.0	2.5	3.3
	16.3	11.9	42.9	28.9	5.6	252	9.0	50.4	16.8	6.7	6.7	5.6	4.1†
1937 Jan.-Feb. March-May June-Aug. Sept.-Dec.	9.2	19.8	28.2	42.8	2.3	131	13.7	28.2	32.1	3.8	13.0	2.3	6.9
	15.4	9.6	37.2	37.8	1.9	156	8.3	30.8	34.0	9.6	7.7	5.8	3.8
	13.8	10.6	44.7	30.9	3.2	123	7.3	25.2	22.7	26.0	10.6	3.2	4.9
	12.3	13.5	56.8	17.4	3.1	229	10.0	23.1	46.3	10.9	3.1	2.2	4.4
1938 Jan.-Feb. March-May June-Aug. Sept.-Dec.	13.33	11.1	61.5	14.1	4.4	135	5.2	17.0	63.0	8.9	3.0	0.7	2.2
	12.67	15.5	62.6	9.2	2.1	142	4.9	28.2	54.9	5.7	2.8	0.7	2.8
	16.9	25.4	52.1	5.6	7.0	71	7.0	8.5	63.4	15.5	1.4	4.2	...
	16.3	20.3	59.5	3.9	5.9	153	9.8	23.5	53.0	7.8	0.7	3.2	2.0
1939 Jan.-Feb. March-May	14.0	14.0	62.7	9.3	6.9	43	2.3	16.3	62.8	14.0	2.3	2.3	...
	14.3	15.8	66.7	3.2	6.3	63	7.9	6.3	79.4	3.2	3.2

* Includes "fatal."

† 0.7 per cent. were Type V.

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Types II (*intermedius*) and III (*gravis*).—The most striking feature is the replacement of Type II as the predominant biological type in Edinburgh by Type III. Type II was responsible for 60 per cent. of cases when the investigation was begun; but, with some periodic fluctuations, the proportion of cases due to this type decreased to less than 10 per cent. in 1939. Type III cases were rare until the end of 1936, rapidly increased in 1937, and in 1938-9 caused 60 to 70 per cent. of cases. For the whole series, the mortality rates amongst cases due to Types II and III were 3.4 and 5.2 per cent. respectively of 473 and 640 cases.



CHART—Percentage Incidence of Cases due to Types II and III *B. diphtheriae*, Case Mortality of Series, and Severe and Moderately Severe Cases.

Type I (*mitis*).—Cases due to this type showed a steady decrease from 24 per cent. in September 1936 to below 10 per cent. in 1939, and were usually associated with a mild clinical form of diphtheria. Only two fatalities occurred in this group, one a child of less than one year of age admitted on the tenth day of the illness, and the other in a post-operative adult case.

Type IV.—This type was negligible until the Spring of 1935 when it suddenly increased in relative frequency. Few cases were due to it in the latter part of 1936, but it increased again in 1937, since when it has not been frequent. It was isolated from 146 cases after March 1936 with a case mortality of 7.5 per cent., *i.e.* higher than the corresponding figure for either Type II or Type III.

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Type V.—Has not been found in Edinburgh since 1936.

Type VI.—The proportion of Type VI strains decreased during the course of the investigation. The highest recorded figure for this type was 15.9 per cent. in 1934-5, but the relative incidence of the type was never higher than approximately 3 per cent. after the end of 1937. From the returns presented in the reports of the Medical Officer of Health for the City of Edinburgh, more swabs were examined for *B. diphtheriae* in 1937 and 1938 than in any year since 1933, and it would thus appear that the chances of isolating this type had not diminished, and that the recorded decrease was not fortuitous.

Atypical Strains.—7 to 8 per cent. of cases were recorded as due to atypical strains in the previous reports, and the proportion has diminished since then. This has probably been due to a tendency to ignore the frequent slight variations met with especially in colony form. It has been noted that it is during the months when cases are relatively few that the colony forms of the various strains tend to lose their type characters. In contrast to this, one's impression is that strains of the predominant type isolated during periods of relative frequency of the disease produce colonies with well-defined type characters.

Notes on Clinical Findings

Severe and Moderately Severe Cases.—The recorded percentage of severe cases does not show any clearly significant fluctuation, though it is noteworthy that one of the lowest figures is for the same period as witnessed a rapid increase in Type III strains (January to February, 1937). The personal factor in the assessment of degrees of clinical severity and the small numbers make the recorded figures of doubtful statistical value; but the aggregate of the severe and moderately severe cases may be rather less liable to error. Taken together these cases showed a sudden rise in 1935 at the time of the increase in the proportion of Type IV cases. Another rise in this curve coincided with the rise in the proportion of Type III cases in 1936-7; but no preponderance of the severer type of case was found in the winter of 1938-9 when the proportion of Type III cases rose from 23 to 64 per cent. The curve of severity rose later but fell in the winter of 1938-9, when the proportion of Type III strains remained high. There were thus three waves of increased severity, that in 1938 being the

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most pronounced; but the significance of such comparatively slight alterations in the curve may be questionable. *The fatality rate* (calculated on active cases and carriers) has on the whole followed the curve for the aggregate of the severe and moderately severe cases, with the possibly significant exception that during the last part of the period under review it showed a tendency to rise. The corresponding figures for the City Hospital were: 1936, 6.9; 1937, 7.0; and 1938, 7.3 per cent.

Carriers. — 42.8 per cent. of strains examined were isolated from carriers in January-February 1937, the highest figure recorded since 1935 (43.6 per cent.), and from only 3.2 per cent. in March-May 1939. Possibly this may be partly due to the difficulty in correctly classifying carriers and very mild cases; but, as stated above, the number of swabs from which the material was drawn did not diminish. The likelihood of finding unsuspected carriers was therefore rather increased than diminished, and it is probable that the drop in the carrier rate, as recorded, is indeed a true indication of an altered carrier state of the population.

Serological Observations

Certain workers in this field have found that the biological types described above are not serologically homogeneous, and the serological sub-types of the *gravis* variety have been especially studied with some interesting results. Agglutination reactions with specific antiserum from individual strains have been used for this purpose. Orr Ewing (1933) recognised four such sub-types and designated them A, B, C and D; Robinson and Peeney (1936) found still another; and the question has arisen as to the epidemiological significance of these sub-types.

A certain number of serological observations have been made with the Edinburgh strains. Agglutinating antiserum for a characteristic *gravis* strain taken at random from among those isolated during September 1938 was prepared according to the method adopted by Robinson and Peeney (1936), and 104 characteristic *gravis* strains isolated in the winter of 1938-39 were tested with it. Bacillary emulsions for the agglutination tests were prepared by the simple technique suggested by Keogh, Simmons, and Anderson (1938) and found satisfactory. The mixtures of bacillary emulsion and serum were left in the water-bath at 55° C. for 18 to 24 hours, as this time was found

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necessary to complete the reaction. Little if any agglutination took place in 4 hours. The floccules were fine but persisted on shaking, and were quite appreciable to the naked eye though the precise end-point of the reaction was often difficult to determine. The great majority of the strains tested reacted specifically with this antiserum, though a proportion (30 strains) exhibited an incomplete similarity to the strain from which the antiserum had been prepared in that the agglutinating titre fell definitely short of the titre of the serum for the strain used for immunisation. Serological observations were also made with antisera for strains of the other types found in Edinburgh, but it is unnecessary to detail these here. The general results indicated that the great majority of the *gravis* strains isolated in Edinburgh during the winter of 1938-39 were serologically similar.

By the courtesy of Professor J. W. McLeod, two *gravis* strains isolated in Leeds during the epidemic were obtained and tested with the Edinburgh *gravis* antiserum. These strains were found to be agglutinated to full titre and also to be capable of completely absorbing the agglutinins for the homologous strain.

Gravis sub-type antisera A, B, C and D (Orr Ewing, 1933; Stuart, 1938) were kindly made available by Dr R. D. Stuart. These had been prepared by him some time previously with type strains supplied by Miss Orr Ewing. Both the Leeds epidemic strains and the representative *gravis* strain were agglutinated by the Sub-type A antiserum, but not by the others; moreover, both the Edinburgh and the Leeds strains were able to absorb the agglutinins from this serum. There is thus conclusive evidence that the majority of *gravis* strains which were predominant in Edinburgh in 1938-39 were serologically *gravis* Sub-type A.

Discussion

The foregoing is a review of the types of *B. diphtheriae* which occurred in Edinburgh from 1932 to 1939. Between 1932 and 1936, Type II was predominant, Type III rapidly increased in frequency at the end of 1936, and in 1937, Types II, III, and IV each accounted for approximately the same proportion of cases. In the autumn of 1937, however, Type III showed a further and greater increase in incidence, and was predominant until the end of the investigation. In the early months of 1939, approximately 80 per cent. of cases were due

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to this type of the bacillus, and, serologically, at least the majority of these were Sub-type A (Orr Ewing). The fatality rate of the series and the corresponding figures for the Edinburgh City Hospital for Infectious Diseases have risen slightly, and the proportion of severer cases has shown a tendency to become greater. On the other hand, the number of notifications has fallen. The carrier rate has also fallen.

Apart from the question of the complete stability of the different biological types of *B. diphtheriæ*, it is now generally accepted that their relative incidence is in a constant state of flux, and varies both from place to place and from time to time (Robinson and Marshall, 1935; McLeod, Orr and Woodcock, 1939); and there can be no doubt that such a change in type incidence has taken place in Edinburgh since 1932.

The significance of this change from one predominant type in particular to another is difficult to assess in the present state of our knowledge. No observations have so far been made to nullify the conclusion that Type I (*mitis*) is usually associated with a mild clinical form of diphtheria; but the data on which this is based cover only some ten years, and it is conceivable that epidemiological conditions might arise which would materially enhance the virulence of this type. Type II (*intermedius*) was the most formidable type found in certain districts according to the records over limited periods of time, as for example in Liverpool (Shone, Tucker, Glass and Wright, 1939); and for some years it was the main cause of diphtheria mortality in Edinburgh. Type III (*gravis*), as the name implies, was the predominant type in the highly fatal epidemics in Leeds, Hull, and elsewhere in England, and it is commonly considered the most virulent type of the bacillus (Cooper *et al.*, 1936). The data collected in Edinburgh, so far, do not clearly substantiate this view, for though Type III was predominant for over a year, there were only slight indications of any increased mortality or morbidity, and the actual number of cases fell. The proportion of the population of pre-school age which had been artificially immunised was estimated at 6.8 per cent. in June 1939, and a similar figure was given for school children. This proportion is usually considered quite ineffectual in face of the onset of an epidemic; and, thus, artificially induced immunity may be discounted as a factor for the purposes of this enquiry. No data are available to indicate the incidence of biological types prior to any of the

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gravis epidemics ; and it may be that in Edinburgh, after a longer period during which the low carrier rate and the case incidence has led to a waning collective immunity of the population, there will be a period of relatively high infectivity of the *gravis* type.

The work of Durand and Guérin (1921), Scott (1923), and Eagleton and Baxter (1923-4) suggested that in one epidemic a single serological sub-type prevails. These workers, however, were for the most part investigating isolated outbreaks and were dealing with comparatively small numbers of strains. More recently, Orr Ewing (1933) found that 10 *gravis* strains from an outbreak in Greenwich Hospital School, a semi-isolated community, were all serologically Sub-type A. Robinson and Peeney (1936) examined 739 *gravis* strains from different parts of the world and found that usually one geographical area was represented by one serological sub-type. They, also, were dealing with quite small numbers of strains from most centres, though 207 were examined from Manchester (205, Sub-type A*), 67 from Huddersfield (all Sub-type A) and 51 from Liverpool (all Sub-type A). From Leeds, Cork, and Hull, places which had recently been visited by severe *gravis* epidemics, they examined 10, 16 and 11 strains respectively, and each place yielded only one serological sub-type (A, B, and C respectively). Eagleton and Baxter, in contrast to their statement that in general a single serological sub-type is responsible for each epidemic, found that their data from two small outbreaks suggested that "though an epidemic may start with one type, others will appear."

In this connection the work done in Edinburgh during an extensive epidemic of scarlatina is interesting. Green (1937) isolated and typed strains of hæmolytic streptococci from 1488 cases over a period of eight months. He found that eight serological types were present throughout the epidemic in varying proportions, and though one was the predominant type at the beginning, it was replaced by another before the peak period. He stressed the importance of examining a sufficiently large number of strains over a sufficiently long period of time in order to arrive at a true knowledge of the serological types responsible for the epidemic. It is possible that this may also be necessary during widespread epidemics of diphtheria.

* Sub-types A, B, and C designated I, II, and III by Robinson and Peeney.

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"*Gravis*, Sub-type A," has been found to have a wide distribution in the British Isles and has been associated with several of the epidemics in England (Robinson and Peeney, 1936); but it is possible that the comparatively small number of cases examined from each centre may have given an erroneous idea of it as one of the main epidemic types. Epidemic strains isolated from Cork and Hull are not "*Gravis* Sub-type A," but B and C.

It seems then unlikely that there is any one biological type of *B. diphtheria* which can properly be considered the epidemic type; and that the serological type which has been associated with severe manifestations of the disease may be predominant in a community in the absence of an epidemic.

Summary

1. The relative incidence of the biological types of *B. diphtheria* in Edinburgh was studied from 1932 to 1939.
2. There was a complete change from Type II (*intermedius*) as the predominant type to Type III (*gravis*).
3. The clinical severity of the cases from which the strains were isolated was correlated with their biological type.
4. No significant change in the clinical form of the disease was apparent during the period under review.
5. Serologically, the majority of the *gravis* strains examined were Sub-type A.
6. The position of *gravis*, Sub-type A, as the supposed epidemic type is discussed.

I wish to express my thanks to Professor T. J. Mackie for his continued interest and encouragement during the investigation; to Dr W. T. Benson and Dr Alexander Joe, Medical Superintendents of the Edinburgh City Hospital for Infectious Diseases, and the Senior Assistants at that Hospital for making available cultures and clinical notes; and to Professor J. W. McLeod of Leeds, and Dr R. D. Stuart, Royal Infirmary, Glasgow, for type sera and cultures.

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