THE TYPES MITIS, INTERMEDIUS AND GRAVIS OF CORYNEBACTERIUM DIPHTHERIAE

A Review of Observations during the Past Ten Years[1]

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During the last fourteen years repeated references to peculiarly severe outbreaks of diphtheria have appeared in the medical journals and in the public health reports for various districts of Europe. One of the earliest of these was from Berlin, where Deicher (37) and Deicher and Agulnik (38) were amazed and chagrined to observe the diphtheria case death rate in the Virchow Krankenhaus rise from 5 per cent in 1924 to 26.7 per cent in the first five months of 1927. Although so high a case death rate has been uncommon, similar observations have been recorded in many quarters. This has been so in a quite special degree in Germany where it is emphasized amongst others by Königsberger (94), Wolter (198), Gundel (64), Paschlau and Sudhues (134), Degkwitz (36), and Woldrich (197). Rostoski (153) points out that in Germany the incidence of diphtheria rose from 30,000 in 1926 to 150,000 in 1936. Goeters (54) states that toxic diphtheria appeared in 1927 in a form which had not been observed for many years and has persisted ever since, while Spörl (168) notes that in Nürnberg there was a higher incidence of diphtheria in 1934 than in any year of this century. Outside Germany, however, there are also records of severe or peculiarly severe diphtheria in Czechoslovakia (45), in the north of France (108), in Italy (19), in Yugo-Slavia (166), in Hungary (198), in the British Isles at Leeds (2, 3), at Hull (98), at Cork (154), in the Ukraine (211), and quite recently in Dundee (178).

Not only are there these numerous records of unusually severe diphtheria, but there is no doubt that in many of these outbreaks the results of serum treatment have been singularly disappointing notwithstanding the great advances in the potency of antitoxic serum. So much is this the case that a considerable

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controversy has gone on in the European medical press on the value of antidiphtheritic serum. Thus Friedberger (49), Zischinsky (213) and Hottinger (82) have expressed marked skepticism about its value in the most severe types of diphtheria, and Paschlau and Sudhues (134), who report a 25 per cent mortality amongst malignant cases treated on the first day of disease, even suggest that in such cases serum is valueless unless administered before the second day. Many observers, however, have rallied to the defence of serum treatment, pointing out that the effect of serum is largely determined by the stage of the disease at which it is administered (6, 153, 188). The evidence for the value of serum and especially of its early administration can scarcely be questioned.

The skeptics have not, however, been easily silenced and reference has been made to Bingel's earlier work (1918) based on a series of over 900 cases in four years in which normal horse serum was found equal to specific antiserum in treatment. Lastly, it is suggested that the argument about superiority of serum being proved by contrast of results from early and late administration is not entirely conclusive, since higher death rates appear in cases admitted late quite independently of any serum therapy (70). On the other hand there are records of very large series of treated cases with extremely low deaths as *e.g.* Hecksher (74), who cites 4819 cases in Copenhagen with only 1.2 per cent of deaths after excluding 11 cases admitted moribund; and Widowitz (193), who records 1112 cases without a death. Von Bormann (14) brings evidence from two different lines of experimental work to dispose of the criticism that normal horse serum is equal to antitoxic sera in treatment of diphtheria. The first is that when these are tested in parallel experiment on diphtheritic infections of the guinea-pig's conjunctiva, it is found that there are three grades of infection varying with the dose administered: mild, moderate and severe. No distinction can be drawn between normal horse serum and antitoxin in the treatment of the first and last but quite a marked one in the group of animals receiving a moderately heavy injection. This difference is shown both in preservation of life and in healing of the eye intact. Not only is this the case, but if the antitoxin contents of various "normal" horse sera are titrated as well as their protective activities in curative experiments a direct ratio of antitoxin content to protective value is established.

There are, therefore, on the one hand observers of competence expressing skepticism about the value of standard diphtheria antitoxin in severe toxic diphtheria, founded on series of cases which are large enough to be significant, and on the other many series of cases in which the value of serum is clearly established, particularly when given early. What does this all amount to? There seems to be an unwillingness in some quarters to accept what is obviously the most probable explanation, *i.e.* that diphtheria in one place is different from what it is in another and that in the same place it may change over a period of years; and that however clearly established it is that over all cases serum is highly valuable, there may still exist a variety of diphtheria in which its effect is at best disappointing. Thus we have Leete, *et al.* (98) in England, Zischinsky (213) in Austria, Schiff and Werber (155) and Paschlau (132) in Germany and Anderson, Goldsworthy and Ward (4) in Australia all describing cases which

have died notwithstanding large doses of serum on the first day of disease. *This must mean that with certain varieties of diphtheria in certain patients it will always be impossible to give serum in time to be of use—that is so long as we are limited to the forms of antiserum now available.*

There are two other aspects of diphtheria as it has appeared in Europe in the last ten years, in which a change from the diphtheria general at the beginning of the century has been noted by most observers. These are a shift of the main incidence to school children from those of the age group 1 to 5 years, and a marked diminution of laryngeal diphtheria.

Age incidence. The evidence of the shift of the incidence from the infant group to that of the school children comes from all quarters in Germany and is very consistent. Thus in Hamburg, Bamberger and Lachtrop (7) observe a change about 1927. Before then there are two or three cases amongst very young children for every one in children of school age; but after 1927 the majority of cases occur in children of six years and above. Behr (9) at Plauen i/V observes the same rather later, 1933. In Lübeck the same shift occurs from 1927 onwards (54). Rostoski (1938) in a wide survey refers to increased incidence in the 6–14 years age group. In the Ruhr the incidence in children over 6 is three times as great as in those below it in the period 1934–36 (183). Spörl (1936) in Nuremberg observes that diphtheria mortality in the 1900–09 period was negligible above 9 years, whereas in 1933–35 it was maximal in the 8–9 years group and considerable in older children. Zischinsky (212) in a very extensive survey of diphtheria in Vienna from 1903–32 shows that in 1922, a year representing the lowest point in the curve of diphtheria incidence, the disease was still mainly in the 0–5 years group as in 1903, but that from 1926 onwards the main incidence moved to the older children. Similar observations have been made in Great Britain (35, 203); and in a recent comprehensive survey of diphtheria in Liverpool, Wright (204) records that in the period 1937–40 more than two-thirds of the diphtheria has been in children over 4 years of age. Cheeseman, *et al.* (20) have made an extensive statistical investigation of the possible causes of this shift of age incidence and are inclined to explain it on the grounds of decreasing congestion of the population dependent on the falling birth rate and general amelioration of social conditions which they argue lead to lessened exposure to contact with infection in earlier childhood and hence the arrival of a larger proportion of susceptible children in the schools. In support of this theory they point out that the high incidence of diphtheria in the younger age group was especially a phenomenon observed amongst the poorer classes in the community. This explanation, however, does not take into account the rather sharp coincidence between the appearance of the more toxic forms of diphtheria and the rise in age incidence which is a feature of so many of the reports from central Europe. It may well be, of course, that two or more separate factors have been responsible for this result.

Diminishing incidence of laryngeal diphtheria. It is obvious that since cases of obstructive diphtheria have always been more frequent in infants, any advance in the usual age for contracting diphtheria will automatically reduce the

number of laryngeal obstructions. When, therefore, there is talk of a decrease of laryngeal diphtheria which has coincided with an advance in the normal age incidence of the disease, it is important to determine whether the decrease in laryngeal diphtheria exceeds that which would naturally follow from change in age incidence.

Bamberger and Lachtrop (7), who devote a paper specially to the decrease in laryngeal diphtheria, bring forward figures which leave no doubt on this subject. In the 0–6 years group with a decrease of about one-third in total cases of diphtheria there is a five- to six-fold decrease in laryngeal involvement between the periods 1927–36 and 1917–26. In the older group, above 6 years, this is still more marked since with a two-fold increase of total diphtheria incidence, laryngeal diphtheria becomes about half as frequent as before. These writers also record that the decrease is not so much in the primary laryngeal as in the combined laryngeal and pharyngeal diphtheria.

Similarly Spörl (168) observes in Nuremberg a fall in laryngeal diphtheria from a maximum of 85% of all diphtheria in 1907 to less than 5% in 1934. It is altogether unlikely that the shift of diphtheria from one age group to another was adequate to account for this. Zischinsky (212) in Vienna records 37% of croup complicating diphtheria in 1903, 28% in 1922 and 13.2% in the period 1926–32 in the 0–4 years group of cases. It is therefore sufficiently clear that laryngeal diphtheria has decreased independently of the change in age incidence of diphtheria.

In contrast to all this, it is undoubted that in Denmark there had been a steady decline in mortality from diphtheria for many years up till 1931 (5). The contrast with North America is still more striking, for not only has diphtheria been nearly obliterated in big cities like New York, Toronto and Montreal, (102, 46, 47, 159) where comprehensive campaigns in prophylactic inoculation have been carried through successfully, but there has been a marked fall in the incidence of diphtheria throughout the country. Figures given in the *Journal* of the American Medical Association, 1938 p. 524, show that over the whole country there has been a drop in diphtheria mortality per 100,000 of population from figures which varied from 7 to 38 twenty-five years ago, to figures ranging from 0 to 6 in 1937 and that for a group of 88 cities with a total population approximating to 40,000,000 the rate had fallen from 10.84 in 1924 to 1.46 in 1937. Schumann and Doull (157) in discussing the phenomenon of decreased diphtheria mortality in Cleveland in particular and in other large cities of America recognize of course the part played by protective inoculation but doubt if that in itself adequately explains the phenomenon. They quote Frost's summary of this matter in the following terms: "One of the three changes tending to reduce morbidity, the decreased portion of Schick positives, may reasonably be attributed to artificial immunization. The other two more important factors, i.e. diminished infection frequency and the smaller ratio of cases to infections are not related in any obvious way to artificial immunity".

It is abundantly clear from consideration of the foregoing records that in the last twenty years there has sprung up a marked contrast between the incidence and severity of diphtheria in North America on the one hand and in many parts of Europe, and especially central Europe, on the other. What is the explanation of this contrast? Does it depend entirely on the more enthusiastic adoption of prophylactic inoculation in the New World? How far do questions of nutrition play a part? Are there complex and insufficiently understood aspects of the development of mass immunity independent of artificial prophylaxis which come into play or is it determined by weather periods appearing in the shorter 35-year cycles of cold moist weather and the longer 200-year cycles as suggested by Wolter (198)? Lastly may different varieties of the diphtheria bacillus endued with different epidemic potentialities be involved?

It was suggested ten years ago by Anderson, Happold, McLeod and Thompson (3) that there was ground for accepting variations in the nature of the diphtheria bacillus as a significant factor in the development of severe diphtheria in epidemic form. On the basis of a limited local experience it was shown that two distinct varieties of the *Corynebacterium diphtheriae* might be distinguished: *C. diphtheriae* type *gravis* and *C. diphtheriae* type *mitis*, the former more prone to produce toxic or malignant diphtheria and liable to epidemic spread, the latter associated more usually with mild and sporadic cases and dangerous to life chiefly on account of obstructive phenomena. Observations in a wider field soon made it clear that there was a third cultural group of diphtheria bacilli sharply defined from the others, often associated with severe toxic cases of diphtheria but less liable to epidemic spread than the *gravis* type and for this variety which has been very specially studied by Mair (104) and to which attention was drawn by him at the outset of these studies the designation "*intermedius*" has been suggested. In the last eight years this suggested classification has been a subject of study and controversy in all parts of the world and many new media have been introduced to facilitate the differentiation of these types (28, 69, 119, 170, 171, 176).

It is now possible therefore to make a definite statement about what is generally accepted with regard to the existence, nature and distribution of these types. There is, however, a good deal of diversity of opinion about their significance, and it will be necessary therefore to state the basis for such conflict of opinion as there is on the subject.

EXISTENCE, NATURE AND DISTRIBUTION OF THE GRAVIS, INTERMEDIUS AND MITIS TYPES OF THE DIPHTHERIA BACILLUS

The existence of these types has been so widely recognized and accepted that it cannot be considered to be any longer in doubt. It has been suggested from time to time, however, (a) that forms diverging from the three chief types described are sufficiently numerous and important in clinical diphtheria to make a wider classification desirable (18, 23, 24, 50, 51, 105, 106, 196, 202, 206).

On the other hand there are many areas in which apart from carriers and a few cases mostly mild all diphtheria has been attributed to one or other of the three types originally described in Leeds (1). Such observations come from northern Germany: Henneberg and Pels Leusden (75) who met only three out of 544 strains which were difficult to classify, and Grossmann (62) who met only five such strains out of 594 (also 26, 64); from Poland (209); from Khartoum (81); from Australia (4, 57, 58); and in England, from Hull (98), from Manchester (150), from Dundee (115), from London (104), and from Liverpool (204), where the most extensive of all recorded investigations of this kind has been carried out. There are a considerable number of observers who while accepting the existence of these types prefer to have them indicated by letters or numbers, as Wright and Christison (202) in England and Hammerschmidt (72) in Germany, who had already (in 1924) described types of the diphtheria bacillus corresponding to *mitis*, *gravis* and *intermedius* in some respects but had not suggested any differences in their pathological significance. This aspect of the question will,

however, be more appropriately discussed under the section on the significance of the types.

CRITERIA BY WHICH THE TYPES SHOULD BE DISTINGUISHED

In a general way, it may be said that where diphtheria has been mild and sporadic observers have found most difficulty in classifying the bacteria which they have handled in the three types *gravis*, *intermedius* and *mitis*, whereas in areas where diphtheria has been severe and epidemic the distinctions have been found easy. Thus in Great Britain atypical strains have been described notably in Stafford (106), in Edinburgh (202), in Glasgow (18) and in Newcastle (173), in all of which areas diphtheria was at the time either mild or only moderately active, whereas in many parts of central Europe where diphtheria has been for a number of years now of considerable severity very little mention is made of atypical strains.

The amount of work which has been done in the U. S. A. on this subject is very limited, the chief contributions being from Maryland (51, 137, 191, 192), from Illinois (63) and from New York City (159). In no case is the *gravis* type described as occurring in even 20 per cent of the cases and insofar as they go the reports suggest that *gravis* diphtheria as it has occurred in Europe has not yet been observed in the U. S. A. The descriptions of the *gravis* strains in Maryland and in New York City, most of which present only some of the characters described for *gravis* strains, suggest that they resemble those observed by the writer over a period of two years amongst material obtained from the Ruchill Fever Hospital in Glasgow. No single typical *gravis* strain was isolated in all this period, although a small percentage of starch-fermenting strains were found which varied in their cultural appearances on solid medium and broth from that of typical *mitis* strains to rougher cultures approximating more nearly to *gravis*. The serious diphtheria in this hospital throughout this period was associated predominantly with the *intermedius* type.

It is fairly obvious also that some observers are recording many more atypical strains than others because they have drawn a large proportion of the strains which they have examined from carriers rather than from cases of the disease, and also because they have insisted on too finely drawn distinctions. It is therefore desirable to define at this stage the criteria which have been found to be most reliable in differentiating these types. These are presented in table 1 in which the characters of primary importance for a rapid differentiation are italicized.

The statements in table 1 under 1, 2, 3, 4, 5 and 7 are based on observations made on many hundred strains gathered in England from Leeds, Hull, Manchester, Liverpool, Newcastle and London; in Scotland from Dundee, Edinburgh and Glasgow; in Wales from Cardiff; in Ireland from Cork; in Germany from Berlin and in Poland from Warsaw (1, 2, 3, 35, and unpublished work.)

The statement under 6 in respect of differentiation between *mitis* and *intermedius* is founded on the unanimous findings of many observers (22, 69, 144, 149, 195, 207). This is so generally recognised that Pesch (138) claimed the development of hemolytic activity in *intermedius* strains subjected to repeated subculture in broth as evidence of their mutation to *mitis*.

The statement under 8 in Table 1 is based upon figures cited in a later section of this review. These observations of the writer and his colleagues were made on a collection of over 300 strains gathered from a wide variety of sources in Great Britain, Ireland and Europe. The existence of considerable numbers of non-pathogenic *mitis* strains is generally admitted (22, 55, 56, 58, 59, 89, 104). The almost invariable pathogenicity of *gravis* strains is also widely acknowledged.

With regard to *intermedius*, the recorded observations are less consistent. In Mair's observations (104) they are the most constantly pathogenic of all types, whereas Wright

TABLE 1

Most reliable criteria in differentiating the three types of C. diphtheriae

	MITIS	INTERMEDIUS	GRAVIS
1. Morphology	*Long forms; metachromatic granules*	*Barred forms often long and clubbed at ends*	Short forms tending to stain uniformly and sometimes closely resembling Hofmann's bacillus
2. Appearance of growth on heated blood agar	Fairly abundant moist, relatively smooth, semi-opaque & glistening colonies	*Flat, fine dry, opaque, and associated with delicate olive green discoloration of medium*	Abundant, flat, dry, matted, relatively opaque
3. Appearance of growth on special blood tellurite media	*Smooth, convex, medium-sized, with black centre and semi-translucent grey periphery for first 36 hours. Finer and larger colonies*	*Flat, fine, dull with black centre and often small central papilla. Grey periphery with slightly raised margin. Colonies very uniform in size*	*Medium to large with slight to marked radial striations and slightly to markedly indented periphery. Color varying from grey-black to black. Finer and larger colonies*
4. Consistence of colonies	*Approximately that of warm butter, colony smears under needle and forms homogeneous suspensions*	Intermediate between *gravis* and *mitis*	*Approximate to that of cold margarine, colony is pushed in front of needle and tends to fracture*
5. Appearance of growth in nutrient broth	*Heavy uniform or mixed uniform and granular turbidity. Pellicle late, soft and leaving ring on side of tube*	*Finely granular turbidity, settling to leave clear supernatant*	*All variations from clear fluid with marked pellicle broken by agitation to coarse flakes which settle to base of tube to slight pellicle over abundant fine turbidity mixed with granules and flakes of varying size*
6. Hemolytic activity on blood agar plates	*Distinct*	*Absent*	Variable
7. Fermentation of starch and glycogen	*Negative*	*Negative*	*Positive*
8. Regularity of pathogenic action in guinea-pigs	10%-20% of non-pathogenic strains (high pathogenicity for mice)	10% non-pathogenic (low pathogenicity for mice and for spermophils)	Non-pathogenic strains extremely rare (moderate pathogenicity for mice)
9. Antigenic homogeneity or diversity	Great diversity of antigenic groups	Antigenically homogeneous	Two main antigenic groups each of which has been found as an epidemic strain over wide areas

(quoted by Stallybrass, 1936) finds about 30% and, Gregory (58) 64% non-virulent, while Rosa (152) observed about 30% of non-virulent *intermedius* amongst strains from carriers. It may be that peculiarities in these strains make the exact technique adopted more important in testing them, and Mair recognised this by using five times heavier suspensions of *intermedius* than of other types in his tests. It is interesting in this connection that they should have been shown to be the least pathogenic of the three types when tested on the spermophil (39) or on white mice (210).

The variations in the reactions of broth produced by the different type strains which were mentioned amongst the differential points in the first communication published on this

subject (3) are rather much influenced by variations in the composition of the medium and of conditions of incubation unless these conditions are very carefully standardized. They are important, however, since the marked tendency to alkalinization of *gravis* cultures may both mask fermentation and interfere with toxin formation.

The statements under 9 in Table 1 are based on the observations of Orr Ewing (49), Murray (117), Mair (104) and especially on those of Robinson and Peeney (151), who have made an extremely comprehensive survey of the immunological types of *gravis* strains.

Atypical Strains. If the suggested subdivision of the diphtheria bacillus into three main groups is accepted, then any strains which present a combination of the characteristics considered important in more than one group will appear as atypical strains. The chief variants from the well-defined types which have been described are the following:

(a) A strain resembling *gravis* in the colony which it produces on differential media such as that of Anderson, *et al.* (3), producing a marked pellicle in broth but failing to ferment starch and glycogen. Such strains have been described under the designation Type IV by Wright and Christison (202).

(b) A very smooth strain producing uniform turbidity in broth but fermenting starch; such strains formed a very small percentage in a series examined by the writer in Glasgow. Carter (18) also found them in Glasgow to the extent of 0.25% in 1600 strains; but 13% of such strains were observed (206) in a small series collected in South Africa, and one or two strains of this kind were found in New York (159).

(c) A strain resembling *intermedius* in colony and morphology but fermenting starch which has been described by Stuart (173).

Types V and VI of Wright and Christison (202) do not seem to require special consideration. They are only differentiated from the others by animal pathogenicity and there would be just as good reason to make non-pathogenic strains otherwise corresponding to *mitis* into Type VII, and non-pathogenic *intermedius* into Type VIII.

Atypical strains are only significant if they appear with sufficient frequency and are found to be definitely associated with serious clinical diphtheria. Judged by these criteria the only one of these forms which requires careful consideration is the rough strain which fails to ferment starch,—Type IV of Wright and Christison.

This variety of the diphtheria bacillus has been found in two of a small group of strains from Cyprus (202); in Glasgow (18) where it was responsible for less than 1% of cases in a large series; to the extent of 4% to 25% in diphtheria in Edinburgh (23, 24); in South Africa where they accounted for 22% of cases (206); in Victoria, Australia (59); in Liège, Belgium, where it was found in 13% of a small series of cases (189); in Leeds by the writer, where it has turned up at long intervals in a group of contacts and in the cases associated with them; and in New York City, where it appears to be not uncommon (159).

Atypical strains have been found in severe cases as well as more frequently in carriers in Edinburgh (23, 24). That they may play a part in significant clinical diphtheria is also evident from the work of Carter (18) who describes two moderately severe cases in Glasgow; and of Block (12) who later described in the same city a severe but limited epidemic apparently due to the consumption of ice cream infected by a carrier in which there were six deaths. From South Africa there were reported (206) three severe cases, one of them fatal, due to such strains.

It is quite clear, therefore, that on the one hand they have been found to be associated with severe and fatal diphtherias and on the other that they are

more common amongst carriers, convalescents and in diphtheria cases of more doubtful significance. Strains of this type—*gravis*-like colony and growth in broth but no fermentation of starch—provide a much larger percentage of non-pathogenic strains than do any of the three main types already described. What then is to be made of them? Are they to be described as a fourth distinct type or are they to be brought within the three main types already described? There are three probable explanations of these strains:

(a) That they are a distinct type as suggested by Wright, *et al.* If so it is one which has as a characteristic a very wide range of pathogenicity. (It is assumed that the only logical classification on the lines of Wright and Christison would be to combine their Types IV and VI as a single type.)

(b) That under this description two forms are being described, one of which is an atypical *gravis* strain which has lost its fermentative activity or developed it only feebly, and the second a form of little or no pathogenic importance. In favor of such a view are the observations of Robinson and Peeney (151) that one or two of the strains sent to them from Germany as *gravis* had lost the power of hydrolyzing starch but were identical in respect of response to agglutinating sera to the main European group of *gravis* strains; also the observation of Keogh, *et al.* (88) that strains of this type met in Victoria resembled the predominant *gravis* strain in Australia in their sensitivity to bacteriophages; and lastly that in Edinburgh the only city in which these diphtheria types have been followed from a period in which *gravis* was practically absent to one in which it was predominant a considerable increase in infections of this type immediately preceded the rapid advance of *gravis* infections (24, 201).

(c) That in each of the three main types there is a rough → smooth variation and that the rough are the more formidable variants in each group. In that case, Type IV of Wright and Christison would represent the extreme rough variation of the *mitis* strain. This theory is approximately that advanced by Clauberg, *et al.* (31). There is much to be said for this explanation, but it does not meet satisfactorily the existence of the large numbers of apathogenic strains of this kind. Extended work is necessary to elucidate the position of such strains.

Stability of the types in culture. The stability of bacterial types may be considered from two sides. It may be studied as a purely bacteriological problem or from the standpoint of the significance of the bacteria concerned in producing disease. Although these are two separate problems they are so far related that if the instability of these bacterial types in culture is shown to be extreme it is a waste of time to consider their significance in connection with disease. If on the other hand their significance in connection with disease is clearly established there may be ground for supposing that the demonstration of mutations under highly artificial conditions of culture is receiving a consideration and emphasis which is not justified. The question of the significance of the different types of the diphtheria bacillus has been recently discussed at great length by Morton (111, 112) and the general conclusion reached that (a) the *gravis* form of the diphtheria bacillus represents the smooth → rough variant in a sequence of changes that can be obtained with the diphtheria bacillus in the same way as they have been

with many other bacterial forms, (b) that a correlation between colony form and polysaccharide fermentation cannot be maintained. The last conclusion is based on the existence of a number of exceptions, and on the fact that Knox and Passmore (93) have demonstrated a limited absorption of oxygen in respiratory experiments in which suspensions of *mitis* strains are prepared in Ringer phosphate with 0.2 to 0.5 per cent of glycogen. The question of correlation of colony form and fermentative activity is clearly one of some importance since it is the point at which most attempts to produce mutations from *mitis* or *intermedius* strains to *gravis* strains have broken down. Thus Christison (21), Carter (18), Cooper, *et al.* (35) and Grossmann (62) have all failed to observe this mutation in collections of cultures kept in some instances for many years. Seydel (162) in a large collection of cultures re-examined after a year in subculture observed one change of *mitis* to *gravis*, which she attributes to a culture initially mixed. It is well that the last possibility should be carefully considered in all rare observations of this kind occurring in large series of strains isolated from mixed bacterial cultures. Gins and Fortner (52) discussing the possibility of variation in diphtheria bacilli emphasize the importance of being on guard against prolonged symbosis of closely related bacterial forms and quote an instance in which specimens of paratyphoid B and *Spirillum alkaligenum* growing in mixed colonies could not be separated even after thirty subcultures from single colonies. Robinson (148), who has devoted much work to this subject, found that one out of 197 *gravis* strains had lost its capacity of fermenting starch on re-examination after 4 to 16 months in subculture, and did not observe any instance of acquisition of the power to attack starch among 395 non-starch-fermenting strains (*mitis* 120, and *intermedius* 275) in the same circumstances. He describes, however, one instance in which the colonies of an *intermedius* strain developed papillae, the subcultures from which after some months of training in starch-containing media gave rise to a starch-fermenting strain. Menton, *et al.* (106) and Leete, *et al.* (98) have also described a single strain in a large series which was atypical and had given rise to both polysaccharide-fermenting and non-fermenting subcultures.

Murray (118) described the transient acquisition of starch-splitting power by *mitis* and *intermedius* strains if these were grown in a rabbit-serum broth. This capacity never survived the first subculture and was obviously related to something carried over from the serum. Carter (18) failed to repeat this observation. Mair (104) is the only observer who has claimed to be able to develop this capacity for fermentation of starch easily in *mitis* and *intermedius* strains. His technique is to heat the cultures just short of the thermal death point and use the subcultures obtained from these. The writer made numerous attempts to repeat these observations but failed and has been unable to find any published corroboration of them.

This may all be summed up in the following sense: no artificial method of converting non-starch-fermenting diphtheria strains to starch-fermenting forms has been described that has been generally accepted. Where very large series of strains have been examined one or two have been detected which have shown

starch-fermenting capacity in a weak or irregular way. Lastly the education of a starch-fermenting strain from one originally non-fermenting has been achieved in one instance by methods similar to those by which in rare instances lactose-fermenting strains have been bred out from non-lactose-fermenting forms like the typhoid bacillus (181).

The observations of Knox and Passmore (93) on which Morton places special emphasis are maintained by him to show that there is no significant difference between the oxygen consumption per unit of dry weight of bacterial suspensions of the type *mitis* and *gravis* when employing glucose or glycogen as substrate! It is true that the amount of oxygen absorbed by the *gravis* strains in these experiments in the presence of glycogen was only some 50 per cent to 60 per cent greater than that absorbed by *mitis* strains in the same experiments, but since in a number of these experiments the controls for some strains showed as much or more oxygen absorption than did others in the presence of glycogen, their interpretation is at least open to doubt. This is the more so as no direct evidence was brought that glycogen was actually destroyed. It should not be overlooked that Passmore (135) in similar experiments got just as good evidence for the use of glucose and glycogen as substrates by *C. hofmannii*.

It would be about as sensible to argue from the latter observation that there was no correlation between glucose fermentation and pathogenicity in the group of corynebacteria as it is to state as Morton does on the basis of the former that there is no correlation between colony form and starch fermentation in the group *C. diphtheriae*. Hohn (80), Schlirf (156) and Warnecke (185) all agree in showing that *gravis* strains produce most acid in starch solutions and that *intermedius* strains produce much less acid in glucose than either of the other types.

Mutations other than those relating to fermentative activities. Many workers have investigated change of type as related to colony form, growth in fluid media etc., and the bacteria have been subjected to a variety of highly specialized conditions for this purpose such as growth in dilute solutions of $CuSO_4$ (142, 61), of LiCl (112), in various concentrations of different animal sera (92). In all of these ways it has been possible to get changes of colony form and of the character of the growth in broth, which are described as being of a more or less enduring character. The simplest of all methods of getting such changes appears to be repeated culture in fluid media at intervals of a week or more and carried on for a considerable period (21); and Pesch (138) claims that *intermedius* strains can be converted to *mitis* with regularity in this way, although he failed both by this and other methods to change *mitis* or *gravis* strains. These methods included culture in filtered sputum, recommended by Weigmann and Koehn (187) as a method by which conversion of type could be obtained in single-cell strains. As, however, these methods have led to conversions of diphtheria strains to *C. hofmannii*, a conversion which rather strains belief, and since the method of controlling the sterility of the sputum which is described is not very convincing, the conversions can only be accepted with some reserve. Lastly, there are quite a number of workers who either fail to observe any mutations at all in the course

of their observations of cultures or only record transient variations from which the bacterium readily returns to its original form as soon as it is allowed to grow in more favorable conditions (42, 61, 63, 75, 92, 144). Grossmann (62) who worked with single-cell cultures in sewage water failed to change *mitis* or *intermedius* but got some change of colony form in *gravis* without change in biochemical activities. Clauberg, *et al.* (31) are also among those who have gone into this problem in a particularly thorough way using single-cell cultures, and they find that all altered strains return to type when transferred to a special serum-cystine medium. They maintain that observations such as those of Christison (21) and Hammerschmidt (72) only amount to rough → smooth variations within three distinct bacterial varieties.

One of the most extensive pieces of work on this theme has been the subject of a thesis in Holland (Siemens, 1938) in which the importance of examining well-isolated colonies is emphasized, a considerable number of atypical forms being brought into the three main groups when so investigated. The general conclusion is reached that *gravis*, *mitis* and *intermedius* represent distinct races and that the numerous variant and atypical forms described are pseudo-types *i.e.* forms of adaptation of the ground type to variations in external conditions. For Siemens, the differentiation of the *C. diphtheriae* to *gravis*, *mitis* and *intermedius* is as fully justified as that of the tubercle bacillus to human and bovine types. Although, therefore, opinion on the subject of the stability of the described types of *C. diphtheriae* is far from unanimous, it may perhaps be said that they have stood up as well to a very fierce flood of investigation as any other recognized bacterial groups. We are therefore examining races which are sufficiently stable and sharply defined in culture in the observations of most workers to enable us to form an opinion about their stability in the human and animal body. From the point of view of their significance in disease this is the most important aspect of the question.

ANTIGENIC GROUPS WITHIN THE TYPES

Work on this subject comes mainly from Ewing (44), Murray (117) and Robinson and Peeney (151). The execution of observations on agglutination of diphtheria bacilli is hampered because of difficulty in obtaining stable suspensions of *gravis* cultures; and a variety of expedients such as prolonged shaking (44) and the use of alkaline solutions instead of saline for preparing suspensions (117, 151) have been introduced. Most attention has been paid by these observers to the classification of *gravis* strains, but they all have recorded a number of observations on *mitis* and *intermedius*. There is good agreement that diversity of immunological groups is most marked among *mitis* strains. The findings about *intermedius* strains are more divergent, as Ewing in a small series finds 2 and Murray in a large one 4 serological groups besides 8 per cent of unclassified strains; whereas all strains of this type examined by Robinson and Peeney fell into one serological group; and the findings of Mair (104) are in accord. It may be that this difference is due to the fact that Murray based his findings entirely on agglutinin-absorption experiments. There is agreement that partial cross-agglutination between the starch-fermenting and non-starch-fermenting groups

may be observed, and Robinson and Peeney mention occasional *intermedius* strains which show considerable cross-agglutination with anti-*gravis* sera and even partially absorb these, an observation which was also made by Carter (18). Ewing, however, did not get cross-absorption, and also points out that the cross-agglutination is not reciprocal, the *gravis* strains failing to respond to *intermedius* serum. Murray found the three types quite distinct when agglutinin-absorption methods were employed.

All observers agreed in finding two or three principal serological groups of *gravis* strains and a few unclassified ones. Murray's groups comprise: (a) strains from Leeds, (b) from Hull and Cork and (c) from Glasgow, and Ewing's (a) strains from the London district and Leeds, (b) from Berlin and Britain, (c) from Hull and London, also 2 strains from Khartoum and 2 from Glasgow which do not fit into these. The work of Robinson and Peeney which is very comprehensive indicates the following groups; (a) strains from Britain and Australia, (b) from Cork, Europe and North America with rare strains in Britain, (c) predominant in Hull and found more rarely elsewhere in Britain, especially London, (d) from Scotland and Egypt, and (e) from Massachusetts only.

The only serious discrepancy in these observations is that Murray places the Cork and Hull strains in one group and Robinson and Peeney place them in different groups. This might be explained by a change of *gravis* strains in Hull, the two observers having obtained their samples at different times. It is to be noted that the Hull strains investigated by Leete, *et al.* (98) are culturally distinct from either the Cork or the Leeds strains. They give a finer, blacker and less rough colony when first plated on heated blood-agar-tellurite medium and they are less inclined to produce pellicle in broth, a finding which favors the observations of Ewing and of Robinson and Peeney rather than those of Murray. Actually Leete found that at a considerably later date (1939) the character of the Hull *gravis* strains had changed over to the typical *gravis* as first described in Leeds, and it was found by the writer that the strains then appearing agglutinated with an antiserum to Leeds *gravis*. This finding was confirmed by Robinson who determined those strains as belonging to his first group. The fourth and fifth groups of Robinson and Peeney do not correspond closely in cultural character to the original description of *gravis*, although they are starch-fermenters. Stuart's (173) unusual starch-fermenting strains from Newcastle were found by him to correspond to Ewing's group B, *i.e.* Robinson and Peeney's Central European group.

In toto these observations may be summed up in the sense that (a) the three types are serologically distinct, (b) each is made up of a number of serological sub-groups, (c) that the *intermedius* group is probably the most homogeneous, (d) that the organisms which fall strictly within the original description of *gravis* can be subdivided into two main serological subdivisions, one widespread in Central Europe and represented in North America, the other widespread in Britain (the recent outbreaks of *gravis* diphtheria in the east of Scotland are of this type), and that both of these have been found to be associated with severe epidemic diphtheria.

STABILITY IN THE ANIMAL AND HUMAN BODIES

The observations on stability in the animal body are in many respects the most reliable which are at our disposal since the bacteria are introduced into parts of the body where no other bacteria are normally present and there is therefore every probability that any bacteria recovered at a later date have been derived from those introduced earlier. Further the conditions approximate more nearly to those which the bacteria are likely to experience in the human body than

TABLE 2

Stability of the types in the guinea pig

OBSERVER	TYPE	NO. OF STRAINS	NO. OF CULTURES RECOVERED*	OBSERVATIONS ON CHANGE
Robinson (148)	*Gravis*	94	243	No significant change
Robinson (148)	*Mitis*	77	144	Two strains showed colonies and broth resembling *gravis*
Robinson (148)	*Intermedius*	111	287	Two strains showed irregularity in colony form, one resembling *gravis*. One strain already referred to is described in detail below†
Cooper, *et al.* (35)	*Gravis*	46	46	No significant change
	Mitis	26	26	No significant change
	Intermedius	13	13	No significant change
	Atypical	7	7	No significant change
Gundel and Erzin (65, 66)	*Gravis*	12	140	No significant change
	Mitis	13	92	No significant change
	Intermedius	7	84	No significant change
Zinnemann and Zinnemann (211)	*Gravis*	1‡		No significant change
	Intermedius	1‡		No significant change

* The number of cultures was greater than strains in some instances because more than one animal was injected with some strains and in many investigations cultures were recovered separately from various viscera.

† The behavior of this strain was very peculiar. It was the one already described yielding subcultures from papillae on its colonies which could be trained to ferment starch. Subcultures from the livers of guinea-pigs injected with this strain fermented starch; those from the local lesion did not unless specially trained.

‡ Passed consecutively through 30 guinea pigs.

those to which they are exposed in cultures. Observations on bacteria isolated at intervals from the human body, on the other hand, are almost always made from surfaces which are both the site of multiple infection and open at all times to implantation of fresh infections from without. The interpretation of changes of type of infecting bacilli on such surfaces unfortunately is a much more difficult matter since it is the question of stability in the human body which is the crux of the whole matter from the practical standpoint.

Observations in the animal body. Table 2 summarizes the observations made in the guinea pig.

Rabbit. Cooper, *et al.* (35) recovered 67 *gravis*, 24 *intermedius*, 47 *mitis* and 11 atypical strains after passage through the rabbit, but they showed no significant change.

Mouse. Pesch (138) passed 6 *gravis*, 6 *mitis* and 6 *intermedius* strains through mice without observing change. Murray (118) injected 16 *gravis*, 17 *mitis* and 16 *intermedius* strains, using 97 mice, and recovered a positive spleen culture from 44 animals. No change of type was observed. Zinnemann (210) passed 10 *gravis*, 16 *intermedius* and 10 *mitis* strains through mice and checked all strains recovered. No change of type was observed.

Rat. Seligmann and Jungeblut (160) were able to effect repeated passage of *mitis* and *gravis* strains in the rat by intracerebral injection without observing change of type.

General. Warnecke (185) also mentions stability of the types in animal passage; and Murray (116) and Siemens (165) resort to special methods, the former injecting strains into animals partially immunized to the same or different types, the latter introducing the bacteria in collodion sacs into the peritoneal cavities of guinea-pigs immunized against various types. Both of these procedures failed to elicit change of type. Nor were Anderson, *et al.* (1933) any more successful in obtaining change of type in the animal body when they resorted to the technique successfully employed by Griffith (60) with the pneumococcus.

Taken *in toto* these observations provide a substantial and remarkably consistent body of evidence in favor of the stability of these types in the animal body.

Observations in the Human Body. It is impossible or at least very difficult to get any conclusive evidence with regard to change of type by repeated examinations of the bacterial flora of the throat, nose, etc. for reasons already stated. There is ample evidence of the appearance of mixed infections and of the isolation of different bacillary types from the same patient at different periods of disease and convalescence. Are these changes to be attributed to cross-infection or to mutation? There is a wide variation in the extent to which change of type in the human body as observed in repeated cultures from the same patient has been recorded.

At Leeds, mixed infections were found (2, 35) to be extremely rare in acute cases of the disease, whereas in convalescents in the wards of fever hospitals they were not uncommon, infections with more than one type or change of infecting type being recognized in 40% to 50% of patients. In the Ukraine, 21% of such changes were observed in convalescents (211). Among 246 cases at Liverpool observed for a month or more in hospital 25% of instances of type change are recorded (53). Gundel (64) notes only a 1% change over all in a large series but a higher incidence of change among hospital cases; and Perry Whitley and Petran (137) in Maryland are exceptional in not observing any change of type on repeated examination. In Königsberg, change was observed in 21% of cases nursed in hospital and in 8% of those nursed at home (142). Kemkes and Steigler (87) found 32% in hospital cases and 10% in home nursed cases. In 19 cases examined in a Manchester hospital over periods up to three months, 11 showed change of type (148). The most extreme instances of type change are given by Hilgers and Thoenes (78), who recorded 85% at Magdeburg.

The fact that about three times as many changes of type have been observed in patients in hospital as in those nursed at home suggests that cross-infection may be a more important factor in such cases than change of type. This impression is borne out by many observations of diphtheria in limited or closed communities. Thus Mittag and Otto (110) describe an extensive although very mild infection of a group of scarlet fever wards in which 45 persons were involved and no change of type was observed over 6 months. Gundel (64) points out that out of 19 small villages in Brandenburg there were 17 in which only one type of the diphtheria bacillus was found. He also points out that where change of type occur in a hospital it is always in wards in which cases of both or all types have been introduced. Preuss (1936) describes an epidemic in a children's hospital of 23 cases all of which were due

to *mitis* strains; and the writer has observed a similar instance of an all-*mitis* epidemic among infants in a maternity hospital. Others (75, 122, 124) also agree that multiple cases in small institutions or in families are all of one type; and it is maintained (122) that where two types occur in one family it is usually due to attendances by children in different schools.

Several British observers have pursued the matter further, attempting to eliminate all possibilities of cross-infection and then observing the effect on the development of type change. "All *Gravis*" wards have been brought under observation in Manchester (148), and Leeds (35), and in these the bacteria disappeared more rapidly than in wards in which mixed cases, *i.e.* cases due to different types, were nursed; and no change of type was observed. Lastly, it was observed (205) over a period of ten months at Liverpool that among 63 cases (34 *gravis*, 12 *intermedius* and 17 *mitis*) nursed in separate cubicles no change of type was encountered. Emphasis is placed on the necessity of repeated examination of the nursing staff in such observations with a view to exclusion of carriers from the wards. Gundel (1936) in Germany also found that there was no type change in wards to which only *gravis* cases were admitted. A report (132) of two cases in which change of type was found although cross-infection could be excluded, was challenged (27) on the ground that the attendants on the patients did not have their throats and noses controlled bacteriologically; this was subsequently admitted (133).

It is apparent therefore that the available evidence is practically uniform in ascribing the occurrence of type change in the human body to cross-infection. Hence the presumption for stability of type in the human body is very strong.

To sum up: a very large number of observers have satisfied themselves about the existence of the three types and of the fact that they are usually readily and sharply defined and further there is much evidence for supposing them to be stable in the human and animal body. It is therefore profitable to go on to summarize the records of their distribution and their associations with clinical diphtheria in various parts of the world.

World Distribution of the Gravis, Mitis and Intermedius Diphtheria Types. There are only three areas in which the quality and amount of the work done is sufficient to give a fairly clear idea of the actual type distribution. These are Central Europe, Australia and Britain. In the remainder, the descriptions of the types published is insufficiently precise or the number of investigations published is too limited to allow of a clear definition of the type distribution.

The data on the geographical distribution of the three types of the diphtheria bacillus have been summarized in table 3.

This wide survey of the work on the types of diphtheria bacilli reveals one or two points of considerable interest. All over the world observers have for the most part found little difficulty in recognizing these types; but in areas where diphtheria is mild and infrequent there may be a rather large number of irregular or atypical forms as in Edinburgh (202); Stafford (106), Capetown (206), Baltimore (50), and New York City (159); whereas in areas visited by severe and epidemic diphtheria the bacterial types are easily and sharply defined: e.g., Berlin (64), Kharkoff (211), and Leeds (3).

A further point of interest is, that with the exception of a short series in Warrington (107) and for the Posen area of Poland (209), no investigation has ever been recorded in which the *mitis* form has not been recognized. On the other hand, there are numerous areas in which the *intermedius* or the *gravis* form either

is not present or is so scantily represented that it escapes observation. Such are: the Sudan (81), South Australia (146), Amsterdam and Rotterdam (165), Posen district of Poland (209), Huddersfield (119), and Helsingfors (120), which

TABLE 3

Geographical distribution of the three types

COUNTRY OR AREA	NO. OF OBSERVATIONS	FINDINGS
Africa (Cape Town, Sudan)	Small	*Mitis* predominant in Cape Town and atypical forms unusually numerous. No *intermedius* in either area (81, 206)
Australia	502	*Gravis* slightly predominant all over; *intermedius* predominant in Melbourne and absent in Adelaide (4, 57, 146, 194)
Canada	Very small	All three types have been recognised (151, 202)
Cyprus	Very small	No *gravis* (202)
Denmark	Very small	No *gravis* (129, 151)
England, Wales	12,282	*Gravis* predominant in Yorkshire 1930–34 and later in Lancashire. *Intermedius* predominant in south and west. *Mitis* predominant in Staffordshire. Fairly even mixture of types in London area, in east Yorkshire after 1934 and in Northumberland (34, 35, 83, 98, 104, 106, 107, 119, 149, 150, 174, 200, 204)
Finland	Small	*Gravis* +; no *intermedius* (120)
France		No information (100, 127, 147)
Germany	4,696	All three strains found in all areas, but *gravis* markedly predominant in east and north-east and to a slight extent in other areas (8, 20, 22, 62, 64, 68, 75, 76, 78, 90, 122, 132, 141, 144, 155, 170, 185, 190)
Holland	521	*Gravis* predominant in Rotterdam, *mitis* in Amsterdam (165, 177)
India	Very small	No *gravis* detected (151)
Ireland (Dublin, Cork)	Small	*Gravis* markedly predominant (35, 40)
Italy	Small	All three types and atypical strains recorded (16, 48, 139, 152, 167, 184)
Poland	841	*Gravis* predominant; *intermedius* absent except in Lemberg (91, 161, 209, 215)
Russia	Small	*Mitis* predominant and *gravis* scanty in Moscow. *Gravis* predominant at Kharkov two years later (163, 211)
Scotland	2,626	*Intermedius* predominant 1934–39 then a swing over to *gravis*; atypical strains numerous in Edinburgh (18, 24, 35, 115, 178, 203)
Spain	Very small	*Gravis* present (25)
Switzerland	Small	All three types and atypical forms (196).
U. S. A.	Small	Typical *gravis* strains rare (50, 51, 63, 79, 137, 151, 159, 191, 202)

have been found to be free of *intermedius* infections; and Edinburgh (201), Copenhagen, New York City and India (151) in which over certain periods at all events *gravis* strains have not been found.

Another noteworthy feature is that there are at least ten of the areas in which investigations have been made that are found to be dominated with *gravis* infection to the extent of 70 per cent or more of all diphtheria occurring. The same has only been observed with *mitis* in Amsterdam (165), and with *intermedius* in Bristol (34), although Glasgow approached closely to 70 per cent of *intermedius* infection in 1932–35. It would seem, therefore, that whereas *mitis* diphtheria is generally endemic, *gravis* diphtheria and to a less extent *intermedius* diphtheria have an epidemic spread. It may be, of course, that what we are witnessing is a gradual spread of *gravis* infection over the world with manifestations of epidemic incidence wherever it appears for the first time.

RELATIONS OF TYPE OF C. DIPHTHERIAE TO CLINICAL SEVERITY OF CASES

The observations which had accumulated on this subject were reviewed by Cooper, *et al.* in 1936; and in a group of about 6,000 cases recorded mainly in Britain and Germany the relative death rates were found to be *gravis* 13.3 per cent, *intermedius* 8.6 per cent and *mitis* 2.3 per cent. Hemorrhagic phenomena appeared to be slightly more common in *intermedius* than in *gravis* infections, whereas paralytic phenomena were definitely more common in *gravis* infections. Paralytic phenomena were less frequent and hemorrhagic phenomena rare in *mitis* infections. The *mitis* strains were, however, responsible for about four times as many cases of laryngeal involvement as the other strains. A summary of the observations which have accumulated since that review are presented in table 4.

From this it appears that in so far as the groups of cases investigated are concerned much higher case death rates have been associated with *gravis* strains than with the others in Australia, Russia and Poland. The highest case death rates are also associated with *gravis* strains in Scotland, in Germany and in the U. S. A., whereas in England in the three districts examined during the period 1936–38 the case death rate from *intermedius* infections has been higher than that for *gravis*. The figure for *mitis* case death rate is for the collected observations much the same as before, falling between 2% and 3% but there are three instances in which an unusually high case death rate has been recorded. One of these is from Australia (4) recording an 8% case death rate. In this instance, however, there appears to have been selection of the more serious cases for detention in the hospital concerned. The other figures from Australia come into line with wider observations on the lethality of this type. The second is from Maryland, where Perry, Whitley and Petran (1936) record a *mitis* case death rate of 8.2%. Unfortunately there are no other records of this kind which would enable us to judge whether that was characteristic of American *mitis* infection or an exceptional happening. In the third instance, the observations (78) at Magdeburg of a case death rate of 19% in *mitis* infection are so completely out of line with all other observations made in Germany and elsewhere that some doubt is left about their validity. This is the more so when it is noted (a) that appearance of colony alone was the criterion for differentiating those strains and (b) that the number of changes of type during the patient's stay in hospital was greater than those recorded in any other series. Preuner (143) makes the interesting observation n connection with these findings that in *mitis* infections the case fatality rises proportionately to the amount of cross-infection in the series of cases. This is not observed with other types.

The second series is rather overweighted with the English observations which amount to more than half of the total, and it is an open question whether a truer picture of the relative severity of each strain would be got by considering all recorded results or by taking a repre-

TABLE 4

Observations on the correlation of clinical severity and type of C. diphtheriae infection recorded since earlier results were summarized by Cooper, et al. (1936)

AREA	REF.	GRAVIS			INTERMEDIUS			MITIS			ATYPICAL		
		Cases	Deaths	%	Cases	Deaths	%	Cases	Deaths	%	Cases	Deaths	%
Australia	(4)	96	25					88	7				
	(57)	12	1		25	1		12	0				
	(194)	109	5		13	1		47	1		31	0	
	(146)	32	5					36	2				
		249	36	14.5	38	2	5.2	183	10	5.5	31	0	0
Scotland													
Glasgow	(18)	93	8		1052	58		239	4		80	1	
Edinburgh	(201)	640	33		473	16		153	2		146 (type IV)	11	
		733	41	5.6	1525	74	4.8	392	6	1.5	226	12	5.3
England													
Essex	(174)	253	24		87	12		42	2		17	0	
Leeds	(200)	780	50		157	17		975	18				
	(33)	11	0		447	9		82	2				
Liverpool	(204)	4126	260		1518	162		2395	53				
Huddersfield	(119)	48	3		1	0		5	1				
		5218	337	6.5	2210	200	9.0	3499	76	2.2	17	0	0
Germany	(190)	71	5		37	2		6	0				
	(78)	53	1		17	0		78	15				
	(90)	42	3		82	7		26	0				
	(87)	279	9		124	1		131	0				
	(75)	60	3		41	2		13	0				
	(144)	10	1		19	0		36	0				
	(124)	13	0		16	1		38	2				
	(185)	18	1		7	0		29	2				
	(141)	779	19		139	1		47	0		35	0	
	(143)							200	5				
	(8)	52	9		19	3		14	1				
	(122)	86	3		128	9		155	5				
	(64)	692	54		102	6		37	0				
		2155	108	5.0	731	32	4.4	810	30	3.7	35	0	0
Poland	(91)	55	10					52	2				
	(209)	300	45										
		355	55	15.5	0	0	0	52	2	3.8			
South Africa	(206)	6	0					65	1		27	2	
		6	0	0				65	1	1.5	27	2	7.4

TABLE 4—*Continued*

AREA	REF.	GRAVIS			INTERMEDIUS			MITIS			ATYPICAL		
		Cases	Deaths	%	Cases	Deaths	%	Cases	Deaths	%	Cases	Deaths	%
Russia	(163)	6	1		28	2		64	6		3	1	
	(211)	183	24		2	0		66	1				
		189	25	13.2	30	2	6.7	130	7	5.5	3	1	
U. S. A.	(137)	14	2		101	2		134	11				
	(63)	2	0		48	1		1	0		1	0	
	(159)	3	1					17	1		11	0	
		19	3	12.5	149	3	2.0	152	12	8.1	12.0	0	
Holland	(177)	184	8		94	1		81	3				
	(165)	71	5		37	2		6	0				
		255	13	5.1	131	3	2.3	87	3	3.4			
Totals		11492	926	8.1	6807	487	7.2	6858	181	2.6	663	24	3.6
Per cent of type incidence				44.51			26.36			26.56			2.57

sentative 500-strain sample from each of the larger collections and combining these with similar or approximately similar samples from each of the smaller collections. If the whole of the English results are incorporated, the *intermedius* infections appear to be more severe; if the latter course is followed the *gravis* strains are shown to be responsible for a case death rate twice as great as that of *intermedius*.

A combination of all available observations on the clinical significance of the types, *i.e.* this series together with those published in 1936 (35), gives a grand total of about 25,000 cases. Of these, there are 11,492 due to *gravis*, with 8.1 per cent case mortality, 6,858 due to *mitis*, with 2.6 per cent case mortality, and 6,807 due to *intermedius* with 7.2 per cent case mortality. As judged by case mortality, the *gravis* strains still lead although the *intermedius* strains are very near them, and they are three times as dangerous as the *mitis* strains.

Among observations accumulated since 1936, full clinical details of the cases are only available for those from Leeds and Liverpool which with the addition of a few made in Germany and Hull provide the results shown in table 5.

These results indicate that the *intermedius* type is most severe in respect of hemorrhagic, paralytic and cardiac complications. This is mainly due to the predominance of Liverpool results as *intermedius* infection in this city has been peculiarly severe. If a similar analysis of the cases from Bristol had been available and equal numbers of cases from that area had been included, a result much more like the earlier one would be obtained. This showed that the *intermedius* infection was most often associated with hemorrhagic phenomena but less responsible for paralytic manifestations than the *gravis* strains. This is also the

final result obtained when both series are combined as in table 6. It is interesting that the impression gathered in the earlier series that *intermedius* infection was more often associated with hemorrhagic phenomena is considerably emphasized by the second series.

The Morbid Anatomical Picture Associated with the Different Types of C. diphtheriae. Very little appears to have been done on this subject apart from a series of post-mortem examinations carried out by Clauberg and Plenge (32) and another by the writer with Orr and Woodcock (103). The latter series included 51 cases (11 *mitis*, 14 *intermedius*, 23 *gravis* and 3 mixed) and all macroscopic and microscopic examinations of the viscera were checked by bacteriological investigations in order to determine (a) that the type of bacillus originally isolated from the patient was the one present at death, (b) how far the bacilli had penetrated from the original focus in the upper respiratory tract, (c) whether

TABLE 5

Relation of types to hemorrhagic and paralytic phenomena, myocardial lesions and laryngeal involvement

	PERCENTAGE ASSOCIATION WITH			
	Hemorrhagic	Laryngeal	Paralytic	Cardiac
Gravis	1.2% of 1622	2.5% of 1784	13.1% of 1914	8.2% of 1914
Intermedius	2.1% of 728	2.3% of 859	15.4% of 879	12.4% of 879
Mitis	0.1% of 1758	6.9% of 1845	6.03% of 1890	2.5% of 1890

TABLE 6

	PERCENTAGE ASSOCIATION WITH			
	Hemorrhagic	Laryngeal	Paralytic	Cardiac
Gravis	2.2% of 3118	2.4% of 3066	14.9% of 3648	8.7% of 1988
Intermedius	3.2% of 2175	1.7% of 2090	11.9% of 2358	12.4% of 879
Mitis	0.2% of 2572	7.1% of 2534	5.5% of 2738	2.5% of 1890

secondary infection played any important part in the death of the patient. The examinations were made in Leeds more than two years after a sharp epidemic of *gravis* infection, and during this period there was a falling incidence of *gravis* infection and a rising incidence of *mitis* infection. Apart from a sharp epidemic localized to one school and responsible for six deaths, *intermedius* infections were scanty during this period and the material from such cases was supplemented by specimens obtained through the kindness of Professor H. D. Wright from deaths occurring in Liverpool. There were only three cases in which evidence of cross-infection was obtained. Lesions very similar to those described for *gravis* infections below are recorded by Kroemer (95) in a series of ten fatal cases of toxic diphtheria. The strains were typed in only four cases of this series: three were *gravis* and one was *intermedius*.

The only interesting observations with regard to the possible significance of secondary infection in the observations of McLeod, Orr & Woodcock was the invariable association

of *C. diphtheriae* and *Hemophilus influenzae* in all patchy hemorrhagic lesions of the lungs; these were found most often in *intermedius* infections. No evidence of distribution of *C. diphtheriae* through the tissues has been obtained except that the *gravis* strains have been recovered from areas of the lung most remote from the bronchi with more frequency than other types. Others, however, submit evidence, post-mortem or by blood culture, for the existence of bacteriemia in severe diphtheria (166, 32, 101, 86, 56). In these instances the type, when determined, has been *gravis*. The only *mitis* septicemia described is one noted by Heubach (77) in a mixed infection with streptococcus from which the patient recovered.

There was a definite contrast between the lesions of *gravis* and of *mitis* infections in the following respects: the tendency to damage of the underlying tissues in the area of the local lesion was much more pronounced in *gravis* infection. This was specially noteworthy in the tonsil in which necrosis and hemorrhage was the rule, and the process usually extended to the tissues of the tonsillar bed and to the cervical lymphatics. There was also a reasonably close correlation between the extent of these tonsillar lesions and the amount of focal necrosis present in the lymphoid tissue of the malpighian corpuscles of the spleen. Lesions of the suprarenals were not a feature in this series. In respect of the spleen lesions and absence of suprarenal lesions, Clauberg and Plenge's (32) series agrees. By contrast the deep tissues were relatively normal in *mitis* infections and the reaction consisted mainly in abundant production of fibrinous membrane which extended more often to the larynx and tended to invade the bronchial tree more frequently. These facts notwithstanding, there was little microscopic evidence of penetration of the deeper tissues by the *gravis* strains (cultural evidence was obtained in the lungs as already mentioned) and it would therefore appear that the distinction in lesions was due to a difference in quality or quantity of toxin produced. This conclusion is reached by Kurkhaus (96) who describes similar tonsillar lesions in cases of diphtheria which die early, although he does not go into the question of bacillary types.

These findings are of course in keeping with the universal experience that *mitis* strains are most often associated with obstructive lesions in the air passages. The lesions produced by the *intermedius* strains corresponded in type to those associated with the *gravis* strains but were less pronounced.

Preuner (143) refers to the special connection of severe and fatal cases of *intermedius* infection with bronchopneumonia but does not give any figures on which this suggestion is based. In our series of post-mortems, the bronchopneumonias were more common in the *intermedius* than in the other infections but the difference was not great enough to be significant. An extended series might bring it out more clearly.

There is occasional mention of *intermedius* deaths complicated by bronchopneumonia (63, 155). Wright (204), however, analyzing 475 deaths from diphtheria in Liverpool finds only 2 pneumonia deaths recorded in *intermedius* infections as compared with 8 out of 53 in *mitis* infections and one out of 260 in *gravis* infections. Naturally the *mitis* strains with their tendency to obstructive lesions of the respiratory tract and consequent operative interference are specially prone to pneumonic lesions and five bronchopneumonias are mentioned in the first 16 recorded *mitis* deaths (35). The ground for supposing that the *intermedius* strains are associated with bronchopneumonia independently of obstruction of the upper air passages to a greater extent than the other strains is not therefore definite although it may depend on some special bacterial association which was not active in Liverpool in the period of investigation.

Bamberger and Lachtrop (7) mention a fall in termination of diphtheria cases by bronchopneumonia from 28 in 1925 to none in 1936. Such a change in view of Wright's figures might easily be associated with a turn over from *mitis* to *gravis* diphtheria.

Two recent writers on the pathology of diphtheria (Watjen and Reimann, 1937) without going into the question of types differentiate (a) malignant toxic diphtheria with lesions such as we have described in *gravis* and to a less extent in *intermedius* diphtheria and (b) banal diphtheria in which early death is by obstruction and which corresponds in post-mortem appearances to infections of *mitis* diphtheria.

THE CARRIER PROBLEM IN THE LIGHT OF THE DISTRIBUTION OF BACTERIAL TYPES

The literature on this subject is difficult to analyze because many authors have made no distinction between nasal diphtheria and the carrier estate. Others do not differentiate between contact carriers and carriers in the community at large who have had no direct contact with cases of the disease. The latter distinction is important (26).

Without making these discriminations, however, and by simply gathering up all recorded observations on types of *C. diphtheriae* found in individuals who did not give a history of a recent attack of the disease we get the results recorded in table 7 in which incidences of types in cases on the one hand and in carriers on the other are compared.

In a number of instances only percentages of the different types of carriers are given but when all the actual figures recorded are summed up the result is: 388 or 31.4 per cent of *gravis* carriers, 513 or 47.5 per cent of *mitis* carriers, 146 or 13.5 per cent of *intermedius* carriers, 82 or 7.6 per cent of carriers of atypical strains.

By contrast the figures for the whole series of cases recorded were: *gravis* 45.5%, *mitis* 26.8%, *intermedius* 25.2% and atypical, 2.6%. These combined figures for type incidence, therefore, indicate that the *mitis* and atypical groups are found oftener in carriers than in cases, whereas the reverse holds for the *gravis* and especially for the *intermedius* groups. This is equally clear from observing the detail in table 7. Only 4 out of 17 observers find the *gravis* incidence in carriers distinctly higher than in cases; whereas in one instance only (22) in Berlin is a considerably higher incidence of *intermedius* in carriers than in cases recorded. With *mitis* infection the reverse holds, since 15 out of 17 observers find a higher incidence of that type in carriers than in cases.

Wright (204) is the only observer who has carefully examined the persistence of the various type infections in convalescents in a considerable series of cases in which cross-infections have been excluded. These observations indicate that *gravis* infections are the most persistent and that *intermedius* infections clear up most quickly, whereas *mitis* infections are a little less slow than *gravis* ones in disappearing. These observations would explain satisfactorily the relative scarcity of *intermedius* carriers and the lesser epidemic tendency of this type of diphtheria notwithstanding its severity. They do not however explain the relatively higher *mitis* carrier rate. Of course it may well be that the conditions in Liverpool, in which in fact only a slight excess of *mitis* carriers over cases exist, do not fairly represent the conditions which exist more generally.

Both Mair (104) in London and Hertel (76) in Leipzig describe the *mitis* carrier infection as the most persistent. Hertel attributes this to the greater tendency shown by this type to produce lesions of the antrum and other air sinuses of the head. Clauberg (26) has made the practical suggestion that, as far as isolation of carriers is concerned, we should concentrate on the *gravis* carrier and possibly also the *intermedius* carrier since these are rarer and more dangerous, leaving the more numerous and less dangerous *mitis* carriers alone. He bases this suggestion both on his own observations in Berlin and on that of Dudley, *et al.* (41) that the presence of *gravis* carriers in a closed and immunized community led to a mild outbreak of diphtheria, whereas the presence of *mitis* carriers both before and after this event left the school free from any clinical manifestations of illness. The observation of Otto and Mittag (123) that *mitis* infection ran through 45 patients in a scarlet fever ward without causing any clinical diphtheria is evidence in the same sense. Clauberg's suggestion has been considerably discussed in Germany, where he is supported by Preuner (141) and by Kleinschmidt (90) who records that in a small series of family contacts of diphtheria convalescents one case of diphtheria occurred for every 3 carriers which resulted from *gravis*

TABLE 7

Contrast of type incidence in cases and carriers

PLACE	GRAVIS	MITIS	INTERMED.	ATYPICAL	REF.
Stafford	17	50	0	33 % in cases	(106)
	18.5	55	0	26.5 % in carriers	
Manchester	51.7	28.1	20.2	% in cases	(150)
	30.5	53.4	16.1	% in carriers	
Liverpool	34	41.5	24.4	% in cases	(164)
	34.8	44.7	18.9	% in carriers	
Dundee	6	37	57	% in cases	(115)
	12.5	87.5		% in carriers	
Edinburgh	0	16	50	34 % in cases	(23)
	0	20.2	20.2	59.6 % in carriers	
Berlin	77	10	5	8 % in cases	(22)
	44	32	15	9 % in carriers	
	72	25	3	% in cases	(26)
	28	70	2	% in carriers	
Prussia	79	16	5	% in cases	(68)
	50	29	6	15 % in carriers	
	83	5	12	% in cases	(64)
	Gravis carriers stated to exceed *mitis* carriers				
Königsberg	82	4	14	% in cases	(141)
	64.3	10.2	13.2	% in carriers	(142)
Düsseldorf	15	55	29	% in cases	(144)
	27	53	20	% in carriers	
Leipzig	61	17	22	% in cases	(8)
	80	13.3	6.7	% in carriers	
Berlin	60	27	13	% in cases	(132)
	45	49	6	% in carriers	
Cologne	28	17	55	% in cases	(90)
	24	35	41	% in carriers	
	33	54	13	% in cases	(185)
	0	95.2	4.8	% in carriers	
Belgium	63	18	2	17 % in cases	(189)
	50	50		% in carriers	
Holland	50	22	25	3 % in cases	(177)
	14.3	74.3	8.6	2.9 % in carriers	
Russia	5	60	31	4 % in cases	(163)
	10	90		% in carriers	
Australia	24	24	50	% in cases	(57)
	3	61	36	% in carriers	(58)

contact, one case for every 2 carriers from *intermedius* contacts but only one case for every 12 carriers resulted from *mitis* contacts. In spite of this evidence from his own work favoring Clauberg's suggestion, Kleinschmidt goes over to the majority who conclude (13, 132) against this suggestion, because (a) *gravis* carriers are more numerous than Clauberg supposed, and (b) some workers have recorded a number of severe *mitis* infections and hence this type must be considered potentially dangerous. Clauberg (29) taking up some of these criticisms points out that his suggestion was not put forward as an ideal course but as the best expedient in view of the large number of carriers and the limited laboratory facilities available. The suggestion does not appear to have been discussed directly outside Germany. There is a good deal to be said for it if the *mitis* carrier concerned is not likely to make any considerable contact with children in the 0 to 4 age-group since among these alone does *mitis* cause any significant number of deaths (204).

RELATIVE CAPACITY OF THE DIFFERENT TYPES TO PRODUCE CLINICAL DIPHTHERIA IN SHICK-NEGATIVE AND INOCULATED PERSONS

A considerable number of observations have been made on this subject in Britain but relatively few elsewhere (see table 8). Puckey (146) records a few typed cases of diphtheria in immunized persons in Australia. *Gravis* infections of the immunized are mentioned in Berlin (30), in a country district of Austria (97), in Cork by Saunders and the writer, and very recently by Tulloch in Dundee. In so far as they permit of numerical statement these observations are collected in table 8. This table includes the naturally Shick-negative, those who had become Shick-negative after immunization, and those immunized but not finally Shick-tested.

It is clear from table 8 that there has been no record of significant diphtheria in the immunized and in the Shick-negative apart from the *intermedius* and *gravis* strains; and apart from the experience of Liverpool, where the *intermedius* infections have been particularly severe, diphtheria in the immunized has been dominated by *gravis* infection. Only the accumulation of observations on a larger scale in a number of different centres will finally settle the relative importance of these two types in this respect.

It is, of course, true that one cannot expect to get diphtheria in the immunized from *mitis* strains in an area like Cork where they are rare; but it is also true that in such an area, *i.e.* one with an almost complete predominance of *gravis*, there has been one case in the immunized to every five in the non-immunized population, whereas in Stafford (107) with the highest proportion of *mitis* infection recorded in any large series of observations the cases in the immunized are in the ratio of 1 to 100 in the non-immunized.

RELATIVE PATHOGENICITY OF THE DIFFERENT TYPES IN THE ANIMAL BODY

The techniques followed by the different observers in this field have varied widely and it is not to be expected that their results should coincide exactly. For example Mair, who finds the *intermedius* strain more constantly pathogenic than any other type, always injected five times as much of these strains as he did of the others. In our own observations, on the other hand, in which *gravis* strains were found to give 100 per cent positive results, the combined results of subcutaneous injection in the guinea-pig and intramuscular injection in the rabbit are considered, and 1/4 to 1/8 of the suspension from an 18-hour Löffler

TABLE 8

Cases of typed diphtheria in the Schick-negative and in the immunized population

AREA	TYPE	PER CENT INCIDENCE OF THE TYPE OF DIPHTHERIA IN THE AREA AT THE TIME OF OBSERVATION	SEVERITY OF CASES					REF.
			Fatal	Severe	Moderate	Mild	Total	
Manchester	*Gravis*	52	3	6	7	10	26	(150)
	Intermed.	20			5	4	9	
	Mitis	28						
Stafford	*Gravis*	4						(107)
	Intermed.	20						
	Mitis	68					5*	
	Atypical	8						
Leeds 1931–36,	*Gravis*	77		3	21	41	65	(182)
(mostly 1934–36)	*Intermed.*	10			3	8	11	(35)
	Mitis	13			1	7	8	
Leeds 1938–40	*Gravis*	54		2	15	33	50	(200)
	Intermed.	7				6	6	
	Mitis	39		1	5	29	35	
London	*Gravis*	Not known				14	15†	(131)
	Intermed.	Not known						
	Mitis	Not known						
Liverpool	*Gravis*	36		8		46‡	54	(164)
	Intermed.	25	2	11		24‡	37	
	Mitis	38				17‡	17	
Australia	*Gravis*§	47	1	3		1	5	(146)
	Mitis§	53			1	2	3	
Cork	*Gravis*	91		2	5	9	16	(182)
	Intermed.	4						
	Mitis	4						
	Atypical	1			1		1	

* There is no statement about the severity of these cases.

† 1 not classed clinically.

‡ The figures in the Liverpool group bracketed as moderate or mild have been classified as mild in this summary.

§ Figures include diphtheria in patients with history of previous attacks.

Summary: In all there were 231 *gravis* infections of which 4 were fatal, 24 severe, 48 moderate and 154 mild; 63 *intermedius* infections of which 2 were fatal, 11 severe, 8 moderate and 42 mild; 68 *mitis* infections of which none were fatal, 1 was severe, 7 moderate and 55 mild.

Of the 362 cases of diphtheria occurring in Shick-negative and immunized patients 63.8% were *gravis*, 17.4% *intermedius* and 18.8% *mitis*.

slope culture was injected to each animal. A number of observers again restrict their records to the widely accepted method of the intracutaneous injection of small amounts of bacterial suspensions, as for example Parish (128). Others again (106) have used broth cultures.

It is relevant at this point to ask what exactly it is that is being determined in these tests. For Turewitsch and Kotschetowa (180) the intracutaneous virulence test is essentially a test of capacity to produce toxin *in vitro*. This is probably mainly true, but it seems likely that the results recorded by various workers indicate toxin produced *in vitro* in some cases, *in vivo* in others and most often a combination of both.

Results in guinea-pigs and in a few instances in rabbits; subcutaneous and intracutaneous injection. A general impression of the relative pathogenicity of the different types may be taken from the combined results obtained in Australia, China, England, Germany, Italy, Poland, Russia and the Ukraine (22, 35, 55, 56, 58, 65, 89, 104, 106, 128, 137, 149, 152, 169, 184, 185, 192, 194, 207, 211). Atypical strains: 97 examined, 53.6 per cent found virulent. *Gravis* strains: 2697 examined, 98.1 per cent found virulent. *Intermedius* strains: 2483 examined, 96.5 per cent found virulent. *Mitis* strains: 3634 examined, 84.6 per cent found virulent. Among these observations, series with 100 per cent positive results were obtained in Berlin, Bologna, Hamburg, Kharkoff, Leeds, Manchester, and Maryland with *gravis* strains; in Berlin, Hamburg and Maryland for *intermedius* strains, but are not recorded anywhere for *mitis* or atypical strains. It is noteworthy that the highest figures for virulence in the three main types appear in the series in which the largest numbers have been examined. Two factors which tend to explain this are probably (a) that large numbers of observations are more readily accumulated where an epidemic spread is taking place, and (b) that observers who have recorded large series of tests are more experienced both in typing and in virulence tests.

The general conclusion may safely be drawn from these very extensive observations that on *gravis* and *intermedius* strains which appear to *experienced observers* to be typical, virulence tests are superfluous.

Observations on unusual animals, or by special methods of injection. Rat. Seligmann and Jungeblut (160) explored the method of injection to the brain but got no differentiation among the types.

Mouse. Zinnemann (210), using the technique of intravenous injection to white mice determined the order of virulence to be *mitis* > *gravis* > *intermedius*.

Chinese Hamster. Tung and Zia (179) did not observe differences of pathogenicity amongst the types with this animal.

Spermophil. This animal was found more sensitive than most to diphtheria strains with the exception of the *intermedius* strain to which it was relatively resistant (39).

Method of Conjunctival Injection. Bieling and Oelrichs (10) using this method on guinea-pigs got the following results:

	Death	*Perforation of Cornea*
Gravis strains	5/15	8/22
Mitis strains	0/14	0/22

although in a second series of observations one *mitis* strain caused perforation.

TOXIN PRODUCTION BY THE DIFFERENT TYPES OF C. DIPHTHERIAE AND ON THE CONTROL BY STANDARD ANTITOXIN OF INFECTIONS WITH THESE TYPES

Soon after the *gravis* type of the *C. diphtheriae* was described it was shown by Parish, *et al.* (129, 130) that by comparison with *mitis* strains and much more so Park No. 8, the *gravis* strains were poor toxin-producers *in vitro* and that infections by them were adequately controlled by standard antitoxin. These facts are commonly accepted (84, 85), but they do not cover the whole case, for Murray (116) has shown that it is much more difficult to protect rabbits with standard antitoxin from repeated doses of *gravis* bacilli than from similar doses of the other forms. Then Gundel and König (67) have shown that if animals are actively immunized with various prophylactic agents, the best preparations protect against all strains, but the less satisfactory ones fail or partially fail to protect against *gravis* strains. Further, Gundel and Erzin (66) could successfully treat *mitis*-infected animals with standard antitoxin 24 hours after injection but not *gravis*- or *intermedius*-infected animals; and they showed that passive protection with antitoxin lasted considerably longer for *mitis* infection. Povitzky, *et al.* (140) also showed in a small number of experiments that the control of *gravis* infection with standard antitoxin was more difficult than that of infections with other strains. Etris (43) demonstrated that *gravis* antitoxin was more potent than standard antitoxin in protecting animals against the homologous strain. Still more recently Clauberg (30), in an extensive study of toxins prepared in special media from cultures incubated only 24 hours, in which large numbers of *mitis* and *gravis* strains respectively were used, came to the conclusion that *gravis* toxins were on the average more potent in producing a skin reaction than *mitis* toxins and that their neutralization required more standard antitoxin.

One of the most interesting contributions to the subject and the most recent is that of O'Meara (121) who, working with saline extracts of *gravis* strains, was able to obtain a striking reinforcement and extension of the lesions produced by standard Park 8 toxin by combining sublethal doses of that toxin with the aqueous extract. On the basis of these observations, he supposes the existence of two elements in diphtheria toxin, one abundant in standard toxin tending to kill guinea-pigs in small amounts but producing only slight local lesions, the other slightly represented in standard toxin but abundant in the saline extract of young cultures on solid medium and tending to effect marked local lesions. It is not stated how far this second toxic element can be obtained from *mitis* cultures, and further investigation is necessary to elucidate this very interesting line of work. The observation that 1/50 of a normal M.L.D. of *gravis* strain kills when combined with a subnecrotic dose of staphylotoxin is interesting in this connection (158).

A new aspect of the problem of toxin production by diphtheria strains has been opened up by the work of the Massachusetts laboratories. Pappenheimer and Johnson (126) appear to have been the first to elucidate the role of iron in controlling toxin production as they were working with synthetic media and noted particularly the contrast between toxin production in flasks of soft glass and those of Pyrex glass. Happold (73) noticed that when grown in synthetic media with

a small and optimal iron content, a number of *gravis* strains produced toxin, although much less than the Park 8 strains, and that an interesting feature was that the former as well as *mitis* strains used up the iron content of the medium whereas the latter did not. Mueller (113, 114) has further pursued this work showing that, compared with the Park 8 strain and two others tested, the *gravis* strain was outstanding in its capacity to produce toxin in media rich in iron; and he points to the conclusion which may reasonably be deduced from this that in the conditions existing in the human body of marked iron excess the *gravis* strains may well prove to be the most potent toxin-producers. This is one of the most interesting developments of the study of toxin production by the different types, and it remains to be determined whether it will be found to hold when larger numbers of *gravis* and other strains have been brought under investigation.

All these observations suggest a complexity in the toxic product of diphtheria bacilli which yet awaits full elucidation and which may be specially difficult to define because one of the toxic elements tends to disappear in subcultures on artificial media whereas that studied in the standard preparation of toxin does not.

Feierabend and Schubert (45) in their study of the strains isolated at Prague in a severe epidemic 12 years ago drew attention to a type of strain which administered in doses that did not kill the guinea-pig produced a local lesion which was not controlled by antiserum. This property was lost, however, after repeated subculture. There are besides a considerable number of diverse observations which make it clear that the neutralization of toxin as usually prepared does not cover the whole problem of immunity in diphtheria. Thus on the one hand Parish observed a carrier of the diphtheria bacillus whose serum was completely void of antitoxin, whereas Prochazka (145) mentions a number of cases in which diphtheria had occurred in Schick-negative individuals with quite considerable amounts of antitoxin in their sera. Further, Seligmann and Jungeblut (160) were unable to control with antitoxin the infection of rats' brain which they could set up with *C. diphtheriae*. Lastly, Zoeller (214) discussing the disease as a clinical observer points out the importance of distinguishing between the qualities of receptivity for diphtheria strains and of capacity for reacting to diphtheria toxin.

CONSIDERATION OF THE TYPES IN THEIR SIGNIFICANCE AS EPIDEMIC STRAINS

There has been a tendency in many quarters to deprecate the use of the terms *gravis*, *mitis* and *intermedius* for the different types on the grounds that these terms are misleading since mild or trivial cases of *gravis* infection are observed on the one hand and severe and fatal *mitis* infections on the other (15, 36, 109). No claims that all *gravis* diphtheria is severe and all *mitis* diphtheria is mild have ever been made. The first considerable series of typed cases published (3) showed 144 mild cases of *gravis* infection and 5 severe *mitis* infections. It is true that no death occurred in the first 100 cases of *mitis* infection in that series. What was definitely suggested, however, was the similarity of the very severe clinical diphtheria occurring in Leeds at that time to that repeatedly recorded in Europe in the years immediately preceding; and that it was likely that the type of strain so closely associated with the Leeds epidemic would also be found in connection with the severer manifestations of diphtheria throughout the world; and that in this respect the name of "*B. diphtheriae Gravis*" would be found to be

justified. It was also claimed in this and subsequent publications that the toxic (myocardial, nervous, hemorrhagic) manifestations of diphtheria were specially associated with the *gravis* and *intermedius* strains, while the *mitis* strains were dangerous mainly from their obstructive effects in the air passages. How far has the much wider subsequent range of investigations justified these claims?

Relationship of Types to Epidemic Diphtheria and Severe Epidemic Diphtheria. The nature of an epidemic must and does vary with each infectious disease and it is difficult to find any complete analogy between one and another. The findings already described suggest that diphtheria, like enteric disease, is caused by several closely allied but distinct varieties of bacteria producing very similar but not altogether identical diseases. The spread of diseases of the upper respiratory tract, however, is much less tramelled by measures of sanitary control, and the development of epidemics is probably dependent in part at all events on fluctuations of mass immunity over periods of years. If this assumption is accepted then it would follow that whichever of the types of diphtheria bacillus possesses the greatest power of striking rapidly through the community will also prove to be the one showing the widest fluctuation in incidence, being practically absent in some areas and responsible for 90 per cent or upwards of the diphtheria in others. The figures for incidence of the different diphtheria types in various parts of the world definitely suggest that *gravis* is the type with greatest epidemic tendency and *mitis* that with least, while *intermedius* lies between. It would follow further from the conception of one variety as that with greatest tendency to epidemic spread that if cases are examined in sufficient numbers and in a wide enough area it would be found to be the preponderant type over all. This also is the result obtained for *gravis*. In a total of over 25,000 cases collected from all over the world, it is found to have been responsible for nearly twice as many cases as either of the two other types.

The picture of diptheria, as revealed by the study of type distribution, suggests therefore three closely allied conditions controlled to varying degrees by oscillations of mass immunity in the community, which are not synchronous in different areas; and possibly on all this there may be superimposed long-period cycles of change in the incidence of one or other of the types promoted by weather cycles in accordance with the suggestion of Wolter (198). This to the writer seems a much more probable surmise than that of the appearance of the *gravis* type as a modification of the *C. diphtheriae* not previously encountered.

Three separate cities, where diphtheria has been typed for several years, have each shown a phase of marked predominance of *gravis* infection at some period although these have not coincided in time in the different areas.

In Leeds a period of marked *gravis* predominance, high incidence of diphtheria and high diphtheria death rate has been followed by steady increase of *mitis* infection and a higher *mitis* death rate than previously, synchronising, however, with a general fall both in incidence of and mortality from diphtheria. In Liverpool where a diphtheria, in which at the outset all three types were well represented but *mitis* slightly predominated, was observed for three years there has been a sudden rise of *gravis* to a position of marked predominance at the end of the period of observation. This has been associated with an almost two-fold increase in the number of diphtheria deaths in the city. In Edinburgh, after several years

in which *intermedius* has played the chief part and *gravis* infection has been negligible, there has been in 1938 a marked increase in the *gravis* strains associated with a rise in diphtheria deaths but without any increase in diphtheria incidence.

There is one other area besides Edinburgh in which observations on type incidence have been made over a considerable period of years. These have not been sufficiently extensive and continuous to permit of presentation in the form of a curve. They are of considerable interest. It was shown by Murray (115) in Dundee that there as elsewhere in Scotland the predominating infection was *intermedius*, and *gravis* played a negligible part. The diphtheria death rate per 100,000 at Dundee at this time and for some years before and afterwards ranged from 3.4 to 9. In 1940, however, the incidence of diphtheria in Dundee increased to three times the average for the previous 10 years and the deaths per 100,000 rose to 41.8, a higher rate than in any year since 1890 with the exception of 1925, *i.e.* higher even than in some years of the pre-serum period. This explosive outburst was found by Tulloch to coincide with a gradual rise in *gravis* infection throughout 1940 till in the period December 17, 1940 to February 26, 1941, 47 cases examined showed 89% *gravis*, 13% *intermedius* and no *mitis*. There are a number of other areas in which observations of the incidence of diphtheria types have been made and in which there has been a record of severe diphtheria epi-

TABLE 9

Gravis incidence in areas in which severe epidemic diphtheria has been recorded

AREA	TIME OF OBSERVATION OF TYPE INCIDENCE	PER CENT OF GRAVIS INFECTION	APEX YEAR OF EPIDEMIC	DIPHTHERIA DEATHS PER 100,000 OF POPULATION IN THAT YEAR	REF.
Hull	Nov. 1932–Feb. 1933	59	1932	42	(98)
Warrington	1934	90	1934	49	(107)
Berlin	1933	77	1927–29	13	(22)
	1934	78	1927–29	13	(155)
Kharkoff	Oct. 1936–Aug. 1937	72	Aug. 1936	*	(211)
Stadt Brandenburg	1935	95	1935	*	(64)
Cork	1934–35	91	1930	80	(35)

* Not available.

demics at the time of observation or some years beforehand. The incidence of *gravis* diphtheria in these areas is recorded in table 9.

The associated diphtheria mortality per 100,000 of population is undoubtedly the safest criterion of the menace to the community exerted by any given strain of bacterium, and when the figures for Cork, Warrington, Hull, Dundee and Leeds are compared with (a) that for 88 of the chief cities of America in 1937, *i.e.* 1.46, or (b) the average for England and Wales for 10 years (1929–38), *i.e.* 7.9, it is clear that these places have suffered from epidemics of a very severe order. In all except Hull and Cork, the incidence of *gravis* at the height of the epidemic was about 90% of all diphtheria. In Cork no observations were made at the height of the epidemic, but in view of the persisting 90% *gravis* infection several years later and of similar observations (129) on a small series of strains taken earlier than 1934, it seems altogether probable that this strain was mainly responsible. In Hull where the observations were made as the epidemic commenced to decline, the incidence of *gravis* was only 59% but as in these cases a 19% case mortality was recorded, it again seems most probable that they were responsible for the epidemic.

In Liverpool, where *gravis* has played a considerable and increasing part in the diphtheria of the city since observations on type incidence were started, this strain has developed an epidemic tendency within the last year which has been reflected in the marked rise in the curve of mortality in 1941.

In Berlin the diphtheria mortality per 100,000 has never approached that recorded in some of these smaller centres and this notwithstanding the fact that it was more than quadrupled between 1924 and 1927 (49), and that the extreme gravity of many of the cases (38) has caused much consternation. Here also it seems probable that a *gravis* strain has been responsible although no observations on the types of diphtheria bacilli were undertaken when the epidemic was at its height.

It may well be, however, that a very sharp epidemic passes more slowly through a larger community like Berlin and is waning in one part of the city while rising in another. In such a case the rising mortality in some areas would be partly balanced by falling mortality in others, and an epidemic plateau would be produced as in Berlin in 1927–28–29 instead of a sharp epidemic spike as in Leeds, Hull or Dundee. However that may be and in spite of a higher case mortality rate for *intermedius* than for *gravis* in a number of areas (Liverpool, Romford and Manchester, in 1933), no record has yet been obtained of an area in which *intermedius* has been the predominant strain during a period of epidemic incidence of diphtheria or of high death rate per 100,000 of population (18, 24, 34, 58, 63, 76, 90, 115, 149). In those instances in which figures for death rate per 100,000 in the areas concerned at the time of observation are available, they vary from 14 in Glasgow in 1934 to 3 in Dundee in 1934 and Edinburgh in 1935.

In Stafford (106), in Newcastle (35), in Maryland (137), in Capetown (206), in Amsterdam (165), and in Moscow (163) observers describe diphtheria in which *mitis* is the predominant strain and the diphtheria is definitely mild. In Magdeburg (78), in Lemberg (215), in Halle (122) and in Hamburg (185) diphtheria is also described in which *mitis* is the most numerous strain; but no mention is made of any specially severe associated epidemic diphtheria, although Magdeburg had an unusually high case death rate for *mitis* diphtheria which has already been discussed. In so far as they are available, the death rates per 100,000 of population in these areas with *mitis* diphtheria, the most numerous, range from 0.6 in Amsterdam in 1935 to 8 in Newcastle in 1934.

GENERAL SUMMARY

The existence of three well-defined cultural types of the diphtheria bacillus for which the designations *gravis*, *intermedius* and *mitis* have been suggested has been widely recognized. There are, however, a small percentage of strains which do not correspond closely to any one of these types. The proportion of these atypical strains varies from place to place and is highest where the diphtheria is mild or of moderate severity. The atypical strains are more often found among carriers and convalescents but have more rarely been found to be associated with severe and even fatal illness. They have not been observed at any time to develop an epidemic tendency. The *mitis* strains when they cause death do so mostly in infants owing to obstructive phenomena and pneumonic complications. The *intermedius* strains are very near to the *gravis* strains in the severity of the clinical conditions which they produce although over all the associated case death rate is less than with *gravis* infection and has never reached quite such a high level as the latter in its most severe manifestations. There are, however, many areas in which the *intermedius* case death rate exceeds that due to *gravis*. The *intermedius* strain disappears more rapidly in convalescence, and whether on account of this or for other reasons it has not the same tendency to epidemic spread as *gravis* infection. The outstanding clinical features of severe *intermedius* and *gravis* diphtheria are essentially toxic and hemorrhagic phenomena, myocardial weakness and pareses. The *gravis* strain is outstanding on account of a greater and more constant pathogenicity to animals, a deeper penetration of the tissues in

the human body and a greater epidemic potency. A complete explanation of these points of difference is still lacking.

It seems on many grounds probable that the numerous severe outbreaks of diphtheria described in Central Europe in 1927–37 and found to be specially intractable to serum treatment were due to the *gravis* type of diphtheria bacillus. The question whether the brilliant results of prophylactic inoculation recorded in North America owe their superiority to those obtained in Europe to more comprehensive adoption and better execution or to the absence of epidemic *gravis* diphtheria in the former area still remains to be determined, although the value of this procedure over all has been proved beyond dispute.

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