The diphtheria bacillus / by G.S. Graham-Smith.

Excerpt from
THE BACTERIOLOGY OF DIPHTHERIA.
EDITED BY
GEORGE H. F. NUTTALL AND G. S. GRAHAM-SMITH.

THE DIPHTHERIA BACILLUS

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SECTION IV.

By G. S. GRAHAM-SMITH.

CHAPTER IV.

THE DIPHTHERIA BACILLUS.

Introduction. Morphology of the diphtheria bacillus, in smears and in culture. Size, shape. Staining of the protoplasm. Classification. Involution form. Characters at various periods of growth on serum. Post-fission movements. Branching forms. Spores. Reaction to staining agents. Loeffler's, Gram's, Nicolle's, Neisser's, Crouch's, Coles', Bronstein's, Cobbett's, Piorkowski's, Schauffter's, Falières', Ficker's, De Rovaart's, Peck's, Pitfield's, Andrade's, Pugh's, Higley's, Ljubinsky's, Epstein's and Boni's methods. Cultivation on artificial media. Loeffler's, Lorrain Smith's, Cobbett's, Kanthack and Stephens', Hayward's, and White's serum media. Agar. Gelatin. Potato. Broth. Products of growth in broth. Action on different sugars. Effects of alkalis and acids on the growth. Milk. Litmus whey. Indol production. Vitality in culture. Chromogenic cultures. Influence of temperature on growth. Powers of resistance to cold, drying, light. Period of life in soil and dust. Effects of putrefaction. Action of germicides. Powers of resistance of bacilli enclosed in membrane. Behaviour in food substances: water, milk, butter, cheese, wine, and bread. Virulence. Occurrence of diphtheria bacilli in notified persons, and contacts, in families, amongst attendants, in hospital wards, and schools. Distribution amongst healthy non-contacts. Summary.

Introduction.

The diphtheria bacillus belongs to a group of organisms sometimes spoken of as the Corynebacteria (Lehmann and Neumann, 1896), the members of which resemble one another more or less closely in morphology and cultural characters. Consequently the diphtheria bacillus has to be distinguished not only from species belonging to this group, but from others, which though they resemble it in morphology, differ widely from it in some of their biological characters.

All who have written on the subject of diphtheria bacilli lay emphasis on the great divergence in morphology shown by various strains which in their cultural reactions and pathogenic properties are identical. According to their morphological characters in young cultures the bacilli have been divided into morphological types. Some of these types are common, others are rare. Bacilli resembling the diphtheria bacillus more or less closely in morphology are of frequent occurrence in the throat, nose, eye, ear, skin and genital organs. These have been termed pseudo-diphtheria bacilli.

The diphtheria-like or pseudo-diphtheria bacilli in their various forms have been a source of endless confusion. Some authors have called pseudo-diphtheria bacilli all the diphtheria-like bacilli which they encountered, which did correspond in some of their cultural characters to typical diphtheria bacilli. Others have restricted the term to bacilli which in all points resemble the diphtheria bacillus except in their pathogenic action on guinea-pigs, i.e. non-virulent diphtheria bacilli. Others again, the majority among the more recent writers, restrict the term pseudo-diphtheria bacillus to the organism which will be described in the following chapters as the Hofmann's bacillus.

The subject is still further complicated by the fact that one school of investigators considers that the pseudo-diphtheria bacillus (usually meaning the Hofmann's bacillus) is merely an attenuated and slightly altered diphtheria bacillus, capable under favourable conditions of turning into the diphtheria bacillus and giving rise to diphtheria. The other school hold that this organism is in no way related to the diphtheria bacillus and is incapable under any conditions of being converted into the latter. This is a subject of more than academic interest since the Hofmann's bacillus is commonly present in the normal mouth and nose, and, if the view of the former school is well founded, must have a considerable influence on our efforts to check the disease by the isolation of persons infected with genuine diphtheria bacilli.

Another name, which has been used in various senses and has consequently added to the confusion, is the term Xerosis bacillus. By some it has been apparently used to denote the non-virulent diphtheria bacillus, occasionally found in the throat, while by the majority of writers this name is only applied to a diphtheria-like organism frequently present on the normal and diseased conjunctiva. The relationship of the latter to the diphtheria bacillus has also been a subject of dispute. The term Xerosis bacillus will be here used

only to denote the diphtheria-like organism found in the eye (see Chapter X).

In order to render the subject as clear as possible the diphtheria bacillus and the organisms which resemble it will be considered under the following headings:—

(1) The diphtheria bacillus. Its morphological types, cultural and pathogenic characters and the conditions which influence its growth, vitality, and distribution (Chapter IV).

(2) The Hofmann's bacillus or pseudo-diphtheria bacillus treated in a similar manner (Chapter V).

(3) The relationship of the diphtheria bacillus to Hofmann's bacillus and the arguments for and against the identity of the two organisms (Chapter VI).

(4) Other diphtheria-like organisms which differ in some more or less important characters from diphtheria and Hofmann's bacilli will be considered in the chapter (X) dealing with "Differential Diagnosis." Amongst these is the Xerosis bacillus. The majority of these organisms have been found in other situations than the throat. Consequently it has been thought advisable to consider each in connection with the situation in which it has been found rather than in groups according to their characters. A synopsis of their principal characters is given at the end of that chapter.

Morphology of the Diphtheria Bacillus.

The diphtheria bacillus is most commonly found in the mouths and noses of those who are suffering from clinical diphtheria. It has also been found in the throats of healthy persons who have come into, contact with the patients. More rarely it occurs in lesions[1] of the eye skin, ear, and female genital organs, and on objects soiled by the saliva of diphtheria patients. Very rarely it has been found in milk and water, and a few instances are recorded of animals suffering from the disease.

1. *In smear preparations*[2] *made from false membranes* the diphtheria bacilli stain well by the ordinary aniline dyes and appear as slightly curved or nearly straight rods of various lengths usually about 3μ long,

[1] Examples of the rarer lesions due to the diphtheria bacillus are given at length (Chapter X).

[2] The organisms from which diphtheria bacilli have to be distinguished in such preparations are described later (Chapter X).

but they may be as short as 1μ or as long as 5μ or more. The contour is irregular, and in the majority of specimens at least one end is slightly swollen, giving the organism a wedge or club-shaped appearance. Occasionally both ends are swollen when the bacilli are dumbbell-shaped. This dilatation at the ends is not so marked in specimens seen in smears from false membranes as it is in bacilli obtained from cultures. The ends of the bacilli are usually rounded, but one end may be tapering. Occasionally examples are found with their greatest swelling in the middle and with both ends tapering. The protoplasm is generally irregularly stained and may show more or less transverse banding but some specimens stain uniformly. Darkly stained granules, situated at the ends and occasionally in the middle of the organisms, are frequently present in the protoplasm of the bacilli. When stained by methylene blue these granules show a violet tint. In such smears there is always a want of uniformity in the appearance of the bacilli as they lie side by side, but examples of most of the forms described may be met with in the same film. The bacilli usually lie irregularly scattered or in clusters, the individuals being disposed in all directions, but chains are never found. Some may be contained within the leucocytes. (Pl. XI, fig. 1.)

In *sections of membranes* the position of the diphtheria bacilli varies in different cases, but they are most frequently found in irregular clumps in the spaces between the fibrin in the superficial part of the membrane (Pl. XI, fig. 2). In this situation they may be the only organisms present. On the surface they are often found in very large numbers, but are usually accompanied by numerous other organisms of various kinds. In the deeper parts they are rarely found.

2. *In culture.* The diagnosis of diphtheria is usually made from cultures grown on one of the *serum media*, and descriptions of diphtheria bacilli usually relate to their morphology on these media. In stained preparations made from colonies 12—24 hours old growing on Loeffler's serum medium diphtheria bacilli vary greatly in appearance and may belong to several types. For the sake of convenience and clearness the appearances are described under the following headings: arrangement, size, shape, staining of protoplasm and involution forms.

Arrangement. The bacilli may occur singly, but are more usually arranged in groups of three, four, or more individuals lying side by side at a more or less open angle with each other or occasionally one individual may lie across the others. Sometimes the bacilli lie end to end, but are always at a more or less open angle with each other,

resembling a circumflex accent, or the letters L or V. One small group of bacilli, the members of which are lying more or less parallel, is often found near a similar group forming a mass with interlacing ends. In large collections the bacilli are frequently thrown into irregular interlacing heaps resembling groups of cuneiform characters. The arrangement of the bacilli in the field has also been compared to Chinese letters or pine-needles lying on the ground. Chains even of moderate length are never found.

Size. Diphtheria bacilli from serum cultures vary greatly in length and have been divided according to their size into long, short, and medium length bacilli. The diameter of the bacilli varies between ·2μ and ·8μ and the length usually between 1μ and 8μ, but exceptionally even longer forms up to 13μ occur.

Shape. The individual bacilli are usually slightly curved, but greatly curved forms are frequently encountered. Straight bacilli are seldom met with.

The bacilli are seldom if ever uniformly cylindrical throughout their entire length, but are usually somewhat swollen at one end or the other, and not infrequently at both, especially in the longer forms. Slighter irregularities are generally present throughout their length. The ends are usually rounded, but specimens are occasionally met with in which one end is more or less pointed. More rarely both ends may be tapering.

Diphtheria bacilli may be classified according to their shape and size. Amongst the *shorter* forms wedge-shaped and irregular specimens are the most common. In these forms the swelling usually occupies one half of the bacillus, and is not entirely confined to the end, the other half being distinctly smaller and sometimes having a pointed extremity. Irregular forms with the swelling near the centre, or without any marked swelling are met with amongst these forms. In the *longer* forms wedge-shaped specimens are not common. The organism is gently curved and the ends are usually slightly greater in diameter than the rest of the bacillus, and one or more slight irregular swellings may be present in its length. These bacilli usually have a length of from 3—4μ. A third group consists of bacilli of about the same length or decidedly longer which have either one end or both decidedly swollen. The swollen ends are frequently twice as broad as other parts of the bacilli. Such bacilli are spoken of as *club-shaped.*

Short oval bacilli, which are young forms, may be encountered in almost any culture.

In preparations from any given culture the majority of bacilli are usually found to belong to one of these three groups, the last two of which are of more common occurrence than the first.

Staining of the Protoplasm.

Diphtheria bacilli have also been classified according to the mode in which their protoplasm stains by the common dyes, especially methylene blue. When stained by Loeffler's methylene blue solution, some portions of the protoplasm usually stain deeply and others lightly. The most typical condition is that which is usually met with in the long and club-shaped forms. In these the terminal swellings are usually deeply stained and frequently show in them violet-tinted granules. The rest of the protoplasm in this type is unevenly stained, showing alternate irregular lightly and darkly stained bands. These bands are seldom transversely placed across the length of the bacillus, but cross the long axis of the organism at various angles. Such specimens may be described as (I) *banded* or *segmented* bacilli. Another type of diphtheria bacillus is faintly stained but has irregular patches of darkly stained protoplasm in its substance. These have been termed (II) *irregular beaded bacilli.* A well-marked variety of this type is the form termed by Eyre (1899) the "sheath bacillus." He describes it as showing "a distinct sheath of faintly stained protoplasm tapering to a fine point at either extremity, and enclosing one darkly stained, elongated, lozenge-shaped aggregation of protoplasm about one-third of the length of the entire organism." According to the writer's experience some long irregularly beaded bacilli are always to be found in cultures of the sheath variety. In a third type the darkly stained parts are rounded and disposed regularly along the length of the bacillus, giving it the appearance of a short chain of streptococci. This has been described as the (III) *Streptococcal* form of the bacillus (Cobbett 1901). Of the above three types the first two are by far the most common, and are the ones described by most observers as typical of the diphtheria bacillus in culture. In a fourth type, which is less frequently met with, the bacilli are uniformly and darkly stained throughout their length. If not too darkly stained a narrow lightly staining septum may, however, often be seen in bacilli of this type. These are usually described as (IV) *uniformly* or *solidly* stained bacilli. Lastly (V) *oval* bacilli uniformly stained or with a light central band (very young forms) may sometimes be seen amongst the other forms.

Although oval forms are to be met with in pure cultures of diphtheria bacilli they usually represent only young bacilli (see p. 133), and virulent cultures composed of these forms alone have very rarely been encountered. That they may occasionally, however, be the only forms present in virulent cultures is proved by the experience of Wesbrook, Wilson and McDaniel (1900) during an outbreak at Owatonna.

The darkly stained violet granules (Polar bodies) mentioned above may be met with in all of these types except the very young forms.

Classification of Diphtheria Bacilli.

One of the most satisfactory methods of classifying diphtheria bacilli is that which has just been given, according to their staining properties irrespective of the size of the organisms. By this method five types may be recognised. (Cobbett, IV. 1901, p. 240.)

I. Segmented bacilli. (Pl. VI, figs. 3, 4, 5.)

II. Long, faintly stained, irregularly beaded bacilli. (Pl. VI, fig. 1.) The "Sheath" bacilli belong to this type.

III. Regularly beaded bacilli. Streptococcal forms. (Pl. VI, fig. 2.)

IV. Uniformly stained bacilli, sometimes with narrow median light bands. (Pl. VI, fig. 6.)

V. Oval bacilli, which frequently possess one unstained septum. Young forms.

Many authors have only distinguished between long, medium length, and short diphtheria bacilli, whilst others, using the length of the organisms for their main divisions, have subdivided them according to their staining reactions.

Further Classification of Diphtheria Bacilli.

Wesbrook, Wilson and McDaniel (1900) have introduced a classification which has been very frequently made use of by other observers in describing the bacilli which they have encountered. These authors say "attempts to classify types of bacilli on the appearance of microscopic fields of presumably pure cultures had to be abandoned, and, instead, we were compelled to adopt a classification based on the morphology of individual bacilli. In the study of the morphology of pure cultures in most instances, especially where they have been derived from typical clinical cases of diphtheria, it is the exception to get even a moderate degree of uniformity in the general shape, size, staining

DIPHTHERIA BACILLI FROM SERUM CULTURES.

Fig. 1.
Irregularly beaded form.
Subculture 24 hours.

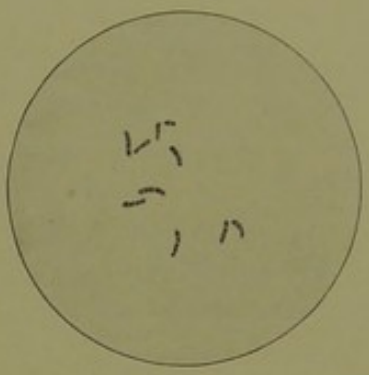


Fig. 2.
"Streptococcal" form.
First culture 24 hours.

Fig. 3.
Segmented form.
First culture 24 hours.

Fig. 4.
Segmented form.
First culture 24 hours.

Fig. 5.
Thin segmented form.
First culture 24 hours.

Fig. 6.
Uniformly stained form.
Subculture 24 hours.

All figures drawn with the aid of a camera lucida (Zeiss $\frac{1}{12}$ im., No. 4 oc.) stained with Loeffler's methylene blue (diluted 1 : 5).

[From Graham-Smith, *Journ. of Hygiene*, Vol. iv. Plate xiv. 1904.]

reactions, etc., of the individual bacilli, whilst to get complete uniformity is not to be hoped for. At first it seemed possible by obtaining an accurate description and coloured sketch of all the bacilli in each pure culture to arrive at a classification. Upon becoming gradually accustomed to close observation, and particularly when it came to sketching the forms, it was soon found that one or more forms might be common to several cultures whose general morphological characteristics seemed to be quite different. By general morphological characteristics is meant the general picture seen by a trained observer. In ordinary routine examinations the observer is influenced perhaps more than he always realises by the presence of certain forms which meet his particular views in regard to type, and may overlook or minimize the importance of other to him less well-known forms which may be present."

The arrangement they adopt is a purely arbitrary and provisional classification of the morphological types (stained with Loeffler's methylene blue) and does not take into account the biological and pathogenic properties of the organisms. Their types are divided into three main groups depending on their staining reactions. (Plates VII, VIII.)

Group I. Granular Forms. These show distinct spherical, ovoid, or markedly rounded granules. These forms are represented by the plain letters, *A* to *G*. In each variety except *B* the granules show metachromatism—i.e. take on a reddish tint with fresh Loeffler's methylene blue. The granules are, as a rule, at one or both poles of the bacilli, though they may occur elsewhere also. The protoplasm of the bacilli not included in the granules varies in intensity of staining, though it is always lighter in colour than, and usually in marked contrast to, the granules.

Group II. Barred Forms. Irregularity of staining is the distinguishing character. Bacilli belonging to this general class present a distinct barred appearance as of actual removal of segments of protoplasm. The darkly staining portions vary in the intensity of the colouration in different bacilli of the same variety and even in the same bacillus. Their size and shape are variable, though, as a rule, not spherical, nor ovoidal, and the colour, whilst usually dark, does not suggest metachromatism. The number of dark segments varies from three to nine. A^1, B^1, C^1, are familiar types of diphtheria bacilli.

Group III. Solid Colour Forms. These include not only the solidly or uniformly staining forms of all shapes and sizes, but also those with the appearance of diplo-bacilli (A^2, B^2, C^2, D^2, E^2, G^1).

Types in Detail.

Types A, A^1, A^2 have often been described as involution forms, but are frequently to be found in smears and fresh cultures. (3—6μ.)

Certain of them, as type A^2, if found as the only microbe present, besides staphylococcus or streptococcus, in a throat culture would not suggest a diagnosis of diphtheria. (Pl. VII, figs. 1, 2, 3.)

Types B, B^1, and B^2. These types are long and slender. (3—7μ.) "*Type B* has been found as almost the sole diphtheria-like organism present in a series of cultures from a case of typical clinical diphtheria and proved to be highly virulent to guinea-pigs." (Pl. VII, figs. 4, 5, 6.)

"*Types* B^1 and B^2 have not been found as the predominating forms in direct throat and nose cultures, though sometimes present in small numbers. It will readily be seen from the resemblance in size and shape of *type* B^2 to various common bacteria of the mouth that it would be very unsafe to diagnose this type as *Bacillus diphtheriae* except when it occurs as a variant in pure cultures."

Type C. Often somewhat curved forms, in which the protoplasm stains light blue except always at the poles and occasionally at one point between the poles, where rounded, rarely ellipsoid metachromatic granules occur (3—6μ). This form is the most common one met with in clinical cases. (Pl. VII, fig. 7.)

C^1 has been frequently met with in clinical diphtheria (3—6μ). (Pl. VII, fig. 8.)

C^2 "Infrequently met with, but up to the present time never found by us as the predominating form even in pure cultures which had long been under study. Its virulence to man and animals is therefore a matter of conjecture" (3—4μ). (Pl. VII, fig. 9.)

Type D. Similar in most respects to C, but shorter and thicker and apparently straighter. (2—3μ.) (Pl. VIII, fig. 1.)

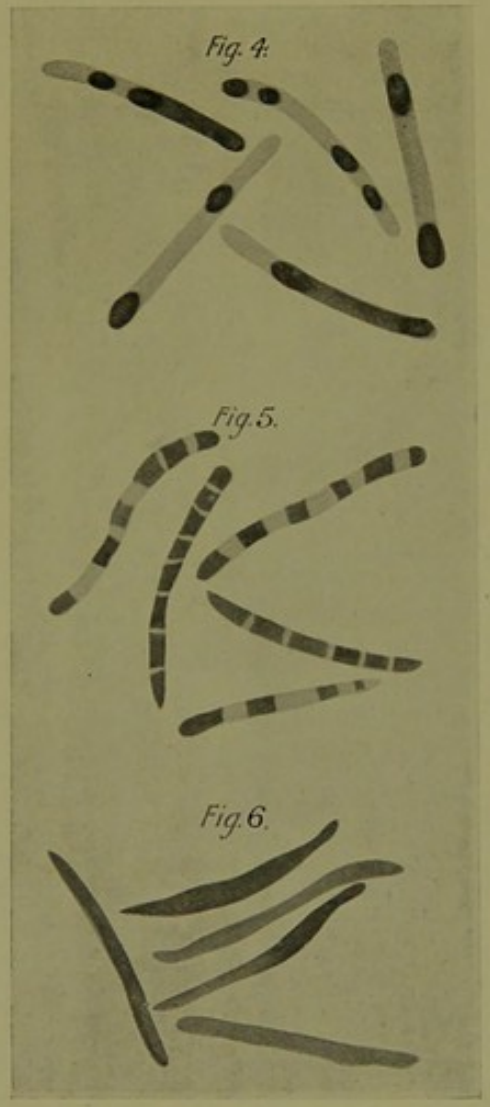
D^1. Shorter, straighter, and with fewer bands than C^1 (2—3μ). (Pl. VIII, fig. 2.)

D^2. Stain an intense even blue. Metachromatism is absent (1—2·5μ). This form probably represents the organism usually spoken of as Hofmann's bacillus. At Owatonna, however, a diphtheria bacillus of this shape was often present alone in clinical cases and was pathogenic for guinea-pigs. (Pl. VIII, fig. 3.)

Type E. Shorter than D, otherwise similar (1—5μ). (Pl. VIII, fig. 4.)

E^1. „ „ D^1 „ „ (1·5—2μ). (Pl. VIII, fig. 5.)

Type E^2. Very like D^2 except that it is shorter and apparently



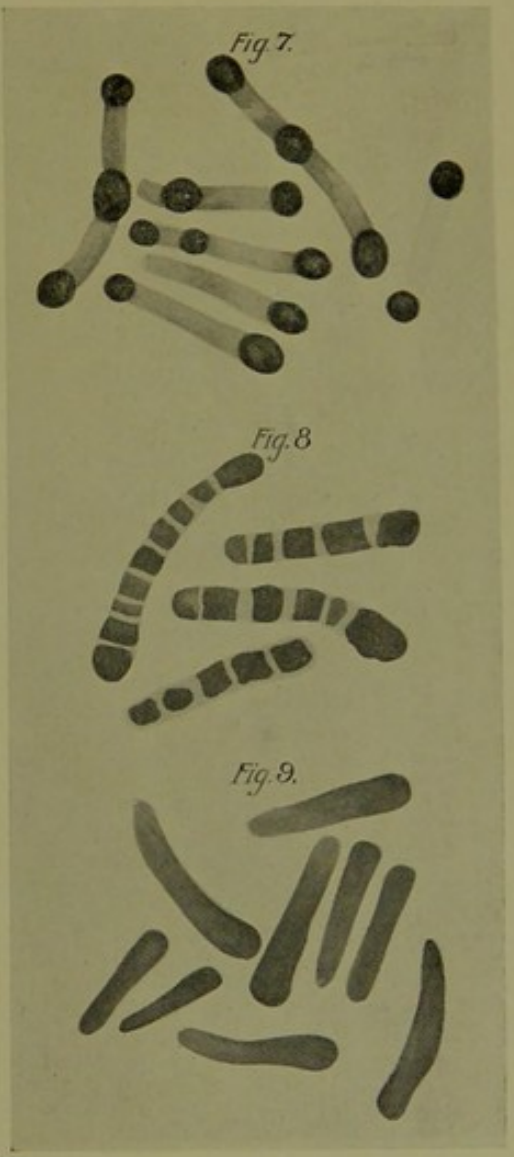
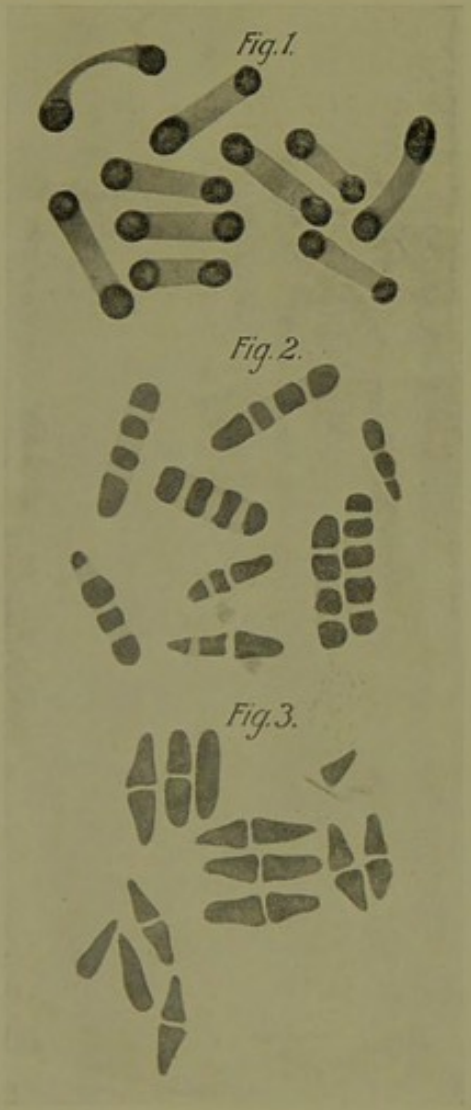


Fig. 1. Type A
,, 2. ,, A_1 } 1 to 2 μ thick.
,, 3. ,, A_2 } 3 to 6 μ long.

Fig. 4. Type B
,, 5. ,, B_1 } 0·5 μ thick.
,, 6. ,, B_2 } 3–7 μ long.

Fig. 7. Type C , 0·5–1 μ thick, 3–6 μ long.
,, 8. ,, C_1, ,, ,, ,, ,,
,, 9. ,, C_2, ,, ,, 3–4 μ ,,

Photographs from Wesbrook, Wilson and McDaniel's (1900) coloured Plates 1, 2 and 3.



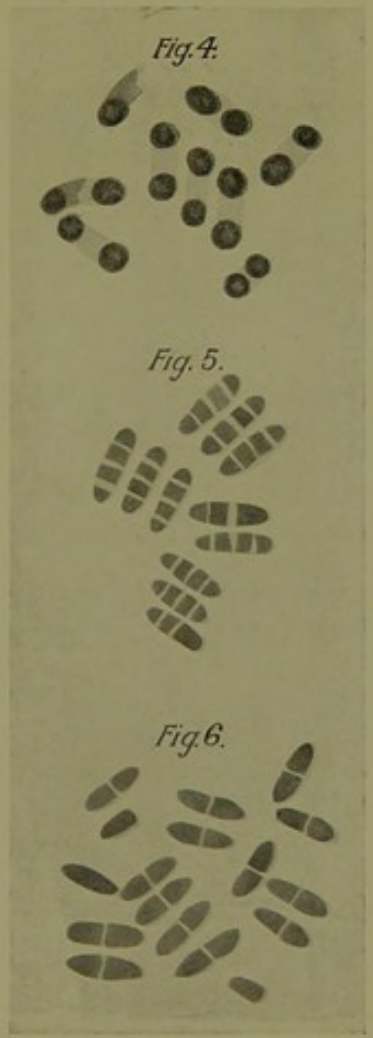


Fig. 1. Type D , 0·75–1 μ thick, 2–3 μ long.
,, 2. ,, D_1, 0·5–1 μ ,, ,, ,,
,, 3. ,, D_2, 0·75–1 μ ,, 1–2·5 μ ,,

Fig. 4. Type E , 0·5–0·75 μ thick, 1·5 μ long.
,, 5. ,, E_1, ,, ,, 1·5 μ–2 μ ,,
,, 6. ,, E_2, ,, ,, 1–2 μ ,,

Fig. 7. Type F , 0·25–0·5 μ thick, 1–2 μ long.
,, 8. ,, F_1, 0·25 μ ,, ,, ,,
,, 9. ,, G , 0·5–0·75 μ ,, 1–1·5 μ ,,
,, 10. ,, G_1, ,, ,, 1–1·25 μ ,,

Photographs from Wesbrook, Wilson and McDaniel's (1900) coloured Plates 4, 5 and 6.

straighter. Probably usually spoken of together with D^2 as Hofmann's bacilli. Not known to be pathogenic (1—2μ). (Pl. VIII, fig. 6.)

Type F. Small (1—2μ) slender bacilli with tapering extremities, stain light blue, and contain one or two metachromatic granules, at one end or near the middle or at both places. "This form is extremely rare, and its pathogenesis has not yet been studied." (Pl. VIII, fig. 7.)

F^2. Like *F* in shape, stains an even light blue, frequently with a central light band. "A rare form, and one which like B^2 would not be recognised as related to diphtheria in throat cultures, but only so when found as a variant in pure cultures" (1—2μ). (Pl. VIII, fig. 8.)

Type G. Shortest (1—1·5μ) granular form, usually with central light band. "The granules are very small, difficult to see and occur only at the distal extremities of the pair. It has been rarely found in clinical cases, though its pathogenesis for guinea-pigs has been shown in cultures from a non-clinical case." (Pl. VIII, fig. 9.)

G^2. A bacillus resembling D^2 and E^2 in staining and arrangement but shorter (1—1·25μ). (Pl. VIII, fig. 10.)

Reference to the observations of Denny on the differences in morphology at various periods of growth and to the observations of Hill and others on the mode of division shows that absolute uniformity of morphology in culture cannot be expected, since the various bacilli in the culture are not of the same age. Nevertheless most cultures have an individuality of their own, which may be preserved for many generations. Moreover it is frequently possible to recognise cultures with marked peculiarities derived at various times from the same individual or from persons infected by him. For descriptive purposes the general impression given by the culture to a trained observer is therefore usually sufficient without the elaborate and confusing tables necessitated by Wesbrook's method of describing a culture by the enumeration of all the various types met with in it.

Another important mode of classifying diphtheria bacilli is according to their virulence for animals (see p. 179).

Involution Forms.

After prolonged growth on a suitable medium, or more quickly on an unsuitable medium, diphtheria bacilli become considerably altered in shape[1]. Their appearance becomes more irregular, and very large forms

[1] The morphological appearances of the bacilli when grown on various media are given together with the microscopical appearances of the growths on these media.

PLATE IX.

Photographs of stained preparations of diphtheria cultures (from Denny, 1903). The magnification in all cases is the same (× 2000), and all the preparations were made from growths on serum and stained with Loeffler's methylene blue (Grübler) 1–3 minutes without heat.

Fig. 1. *B. diphtheriæ*, 5 hours at 36° C. This shows only uniformly staining forms. (Denny, Plate VII, fig. 1.)

Fig. 2. *B. diphtheriæ*, same culture, 8 hours at 36° C. This shows also uniformly staining forms, many of them with parallel arrangement. (Denny, Plate VII, fig. 2.)

Fig. 3. *B. diphtheriæ*, same culture, 15 hours at 36° C. The bacilli stain unevenly and show large granules. (Denny, Plate VII, fig. 4.)

Fig. 4. *B. diphtheriæ*, same culture, 48 hours at 36° C. Shows long forms with large dark staining areas. (Denny, Plate VII, fig. 6.)

Fig. 5. *B. pseudo-diphtheriæ* (Hofmann), 11 hours at 36° C. This shows uniformly staining forms like young *B. diphtheriæ*, but shorter, thicker, and with more rounded ends. (Denny, Plate IX, fig. 11.)

Fig. 6. *B. pseudo-diphtheriæ*, same culture after 24 hours. Comparison with fig. 5 shows diminution in size in the older culture. (Denny, Plate IX, fig. 12.)

Fig. 7. *B. xerosis*, 12 hours at 36° C. This shows only uniformly staining forms. (Denny, Plate IX, fig. 13.)

Fig. 8. *B. xerosis*, same culture after 24 hours at 36° C. This shows the segmented or barred forms which are characteristic of pure cultures of this species. (Denny, Plate IX, fig. 14.)

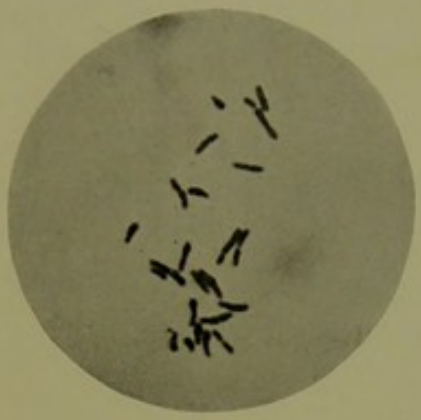


Fig. 1.

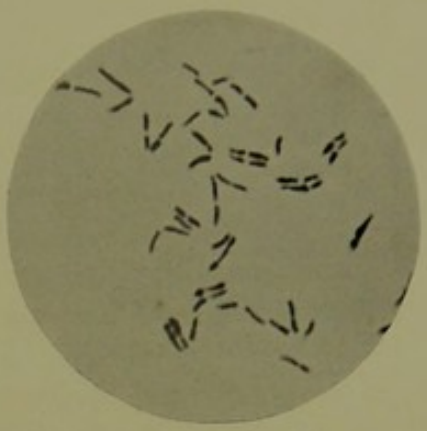


Fig. 2.

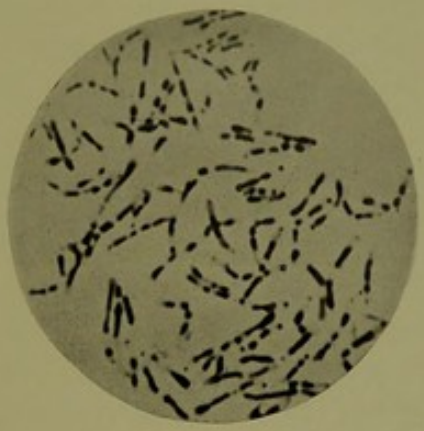


Fig. 3.

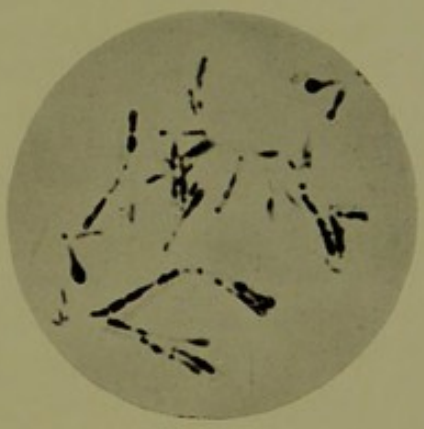


Fig. 4.

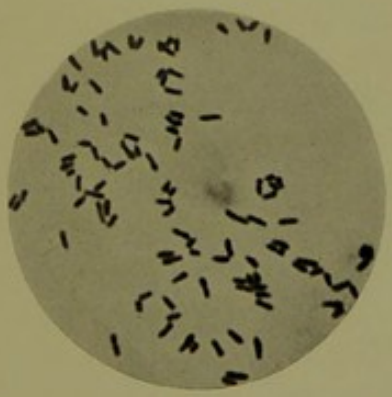


Fig. 5.

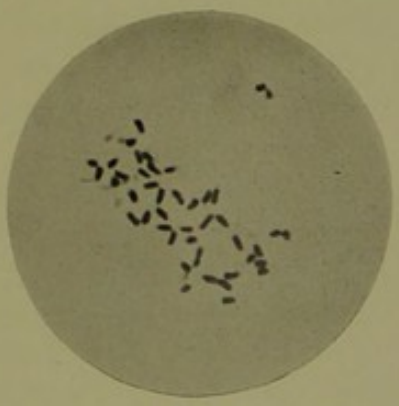


Fig. 6.

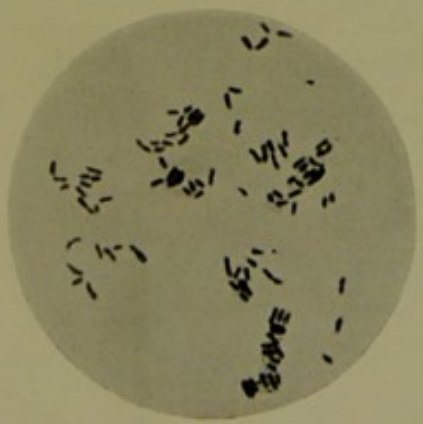


Fig. 7.

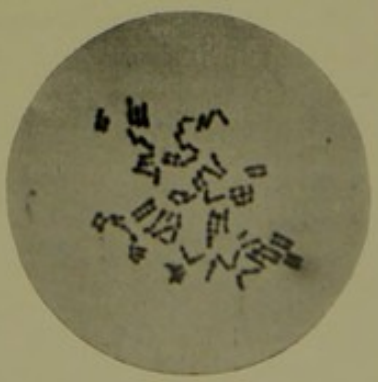


Fig. 8.

are frequently encountered. Many become greatly swollen at their ends and develop enormous club-shaped masses which stain deeply, while the rest of the bacillus may stain badly or have irregular patches of deeply stained protoplasm in it. Others become pear-shaped, or globular, while some retain their general shape but become thicker throughout. Others again show large globules at their ends while the rest of the rod appears as a faintly stained line. Specimens which take up the stain very badly are common. Some bacilli may be represented by small round masses like cocci or by a chain of such masses when they look like streptococci. In fact under such conditions every variety of shape and form and staining capacity may be met with. (Pl. VII, fig. 1, Pl. IX, fig. 4, and Pl. XI, fig. 4.)

Morphological Characters of the Diphtheria Bacillus at various periods of its growth on Serum.

Denny (1903) has recently investigated the morphological characters of 10 strains of diphtheria bacilli at various periods of their growth on serum by means of stained preparations. He inoculated serum tubes and incubated them at 36° C., and made smear preparations from them after 4, 8, 12, 15, 24, 36 and 48 hours' growth.

After 4 *hours'* growth he found the bacilli to be uniformly stained, resembling Hofmann's bacilli in shape, and frequently lying in pairs. There was considerable variation in size and shape, and some of the rods were distinctly curved. At this period they correspond to C_2 and D_2 of Wesbrook's types (Pl. IX, fig. 1).

After 8 *hours'* growth the rods are more uniform in size, still uniformly stained, and arranged in pairs. Small granules or polar bodies are occasionally to be seen in them (Pl. IX, fig. 2).

After 12 *hours'* growth the rods are longer, frequently contain granules, and often have faintly stained portions at their ends. They correspond to the *C* and *D* types of Wesbrook.

After 15 *hours'* growth the bacilli are longer and larger, and segmentation of the protoplasm is moderately well marked. The polar bodies are larger and more abundant (Pl. IX, fig. 3).

After 24 *hours'* growth uniformly stained specimens are few. The bacilli are longer, clubbing is a marked feature, and the majority show polar bodies. Segmentation is well marked.

After 36 *hours* the above appearances are still more marked. The bacilli are longer and many stain poorly.

After 48 *hours* very long forms are numerous and a considerable proportion stain badly (Pl. IX, fig. 4).

Post-fission Movements, studied in Living Cultures.

Hill (1901, 1902) in a series of investigations made observations on the development of living diphtheria bacilli and compared their mode of division with that of various other species. The observations were made by a method which he devised and called the "hanging block" method (1902). Living bacilli are spread on the surface of a sterilized cube of agar, and the inoculated surface is applied to the under surface of a cover-glass. This method allows the organisms to grow horizontally without restriction, and they are all necessarily in optical contact with the cover-glass. Working by this method he has described the post-fission movements of diphtheria bacilli (1901). "Restricting the term fission to the separation of the protoplasm of the bacterium into two portions, each capable of further growth, development, and reproduction, a striking difference as regards fission between *B. diphtheriæ* and its allies on the one hand, and *B. typhosus* (taken for the purpose as a type of a large group distinct from the diphtheroid group) on the other, lies in the post-fission movements. It may at times be possible to detect in *B. diphtheriæ* segmentation of the protoplasm previous to post-fission movements, a hard dark line, not necessarily central, developing across the axis of the rod; sometimes more than one such segmentation line is visible in a single rod. What may usually be observed is a gradual enlargement of the bacterial cell in both diameters accompanied often by slight changes in outline suggesting some plasticity of the bacterial walls; then a sudden snapping across of the rod resulting in the two portions lying at an angle with each other and suggesting for such organisms the convenient term 'snapping group.' Subsequent growth results in the gradual approximation of the distal ends of these two rods to each other, giving a parallel arrangement. The proximal ends of the new rods (those which before snapping were continuous with each other) remain contiguous to each other after parallelism is achieved. In such movements, one or both rods necessarily describe parts of a circle, and when, as often happens, the two rods are not of the same length, the shorter usually moves through a much greater arc than the longer. Subsequent growth does not usually result in the separation of the two portions at the proximal ends, the increase in length in the longer portion apparently carrying the other portion with it. That

something unites the proximal ends seems further evident, because if two rods thus derived be disturbed, as by a current of water, so that they drift off, turning over and over, their relative positions remain unchanged. A single rod may break up into three portions instead of two."

The hypothesis offered by Hill to explain these movements is, in brief, as follows:—"that the visible bacterial rod is surrounded by an invisible or scarcely visible membrane (even in crowded unstained preparations, individuals lying side by side are rarely if ever in visible contact, an interval remaining always between them); that in *B. diphtheriæ* only incomplete rupture of this membrane after fission occurs, the line of rupture running round only a portion of the circumference of the original rod, leaving a bridge connecting the new rods formed; that the enlargement of these rods before snapping and the tension thus produced originates the rupture, and that the preliminary angular position, and the parallelism finally achieved are due to the pressure of the proximal ends of the new rods on each other in composition with the restraint exercised by the unruptured strap-hinge like portion of the original membrane" (Plate X, fig. 1).

The fission and post-fission movements of *B. typhosus* for example are quite different. Hill describes them as follows:—"In this species fission is always clearly evidenced by a sharp separation of the protoplasm of the original rod into two usually equal portions by a translucent segmentation interval. Later a slight curve to one side is observed in one of the two new rods still in line with each other. This curve tends to straighten afterwards, the straightening being achieved by the proximal end of the curved rod slipping slowly out of line with the other rod, towards that side on which was the convexity of the curved rod. The two rods, now free at both ends continue to grow, the proximal ends passing each other in opposite directions, and finally reaching, if nothing prevents, to the distal ends." Species such as *B. typhosus* he terms the "slipping" species (Pl. X, fig. 2).

Plate X, figs. 3, 4 show the development of single specimens of *B. diphtheriæ* into four and eight daughter cells as has just been described. The relations of the various daughter cells to one another and the time which elapsed between the various stages are given.

These movements have also been described by Kurth (1898) and Nakanishi (1901).

Hill and Rickards (1903) investigated the post-fission movements in 18 species of bacteria, namely *B. anthracis, megatherium, coli, typhi, pestis, ramosus, indicus, fluorescens liquefaciens, mucosus capsulatus, pyocyaneus,* the bacillus of logwood, Whipple's *C* and *D* and *Sp. choleræ*

PLATE X.

The figures in this plate are from Hill 1901 (*30th Annual Report of the Health Department of the City of Boston*, p. 81) and are described in his own words.

Drawings made from direct observation of bacilli, developing in hanging block preparations, under the microscope, at 37° C., warm stage. The individuals in each figure (1–4) are drawn to a common scale, but the scale was not the same for all the different figures. The diphtheria bacilli described were derived from cultures obtained in the course of routine diagnostic work of the Boston Board of Health, and were typical in morphology, cultural reactions and virulence. The typhoid bacilli observed were derived from the culture used in the laboratory for the Widal test.

Fig. 1. Actual. *B. diphtheriæ*, illustrating "snapping" (A) became (B) suddenly, the change occurring under the writer's eye; (B) became (C) gradually, as is the rule in this species. Fig. 1. Hypothetical. *B. diphtheriæ*, illustrating the writer's hypothesis of membrane rupture, devised to account for the snapping and subsequently achieved parallelism.

Fig. 2. Semi-diagrammatic. *B. typhosus*, illustrating the definite fission (B), gradual slipping aside of proximal ends (C–D) and subsequent parallelism (E, F), characteristic of this and some other species. This method is illustrated here to serve as a contrast to the snapping of *B. diphtheriæ*.

Fig. 3. *B. diphtheriæ*, illustrating development of a rod (A) into four daughter cells (G); (D) became (G) in 45 minutes.

Fig. 4 illustrates branching by apparent budding. The form (A) gave rise to nine daughter cells (H), of which eight were derived by the ordinary processes of fission, accompanied by ordinary post-fission movements. The ninth and largest cell arose as shown (A–D), by gradual enlargement of the original branch. At (E) the branch "snapped" suddenly to one side on its stem (12); became faint, losing its density and plumpness (F–H). Time relations: A to B = 37 min.; B–C = 32 min.; C–D = 16 min.; D–E = 8 min.; E–F = 62 min.; F–G = 37 min.; G–H = 38 min.; total A–H = 3 hours and 50 minutes.

Fig. 5. A, J–O show branching in the absence of metachromatic granules. G–H show metachromatic granules in the absence of branching and B–F metachromatic granules in combination with branching. In fig. C a granule is situated at the base of a branch. This condition is more frequent than might be supposed from these plates.

The genealogical relationship of the new cells in figures 3 and 4 are indicated by a modification of Ricard's system for culture record (B. R. Ricard, A system of recording cultures of bacteria genealogically for laboratory purposes, *30th Annual Report of the Health Department of the City of Boston*, 1907, p. 75).

Fig. 1.

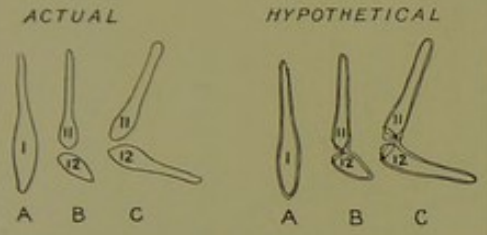


Fig. 2.

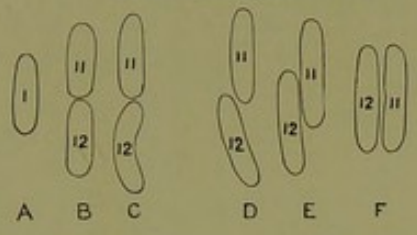


Fig. 3

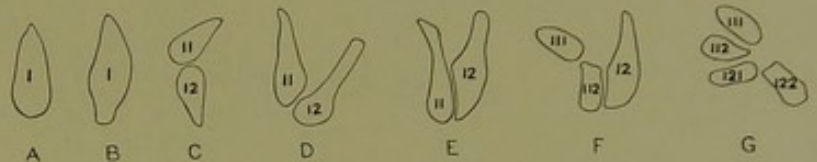


Fig. 4.

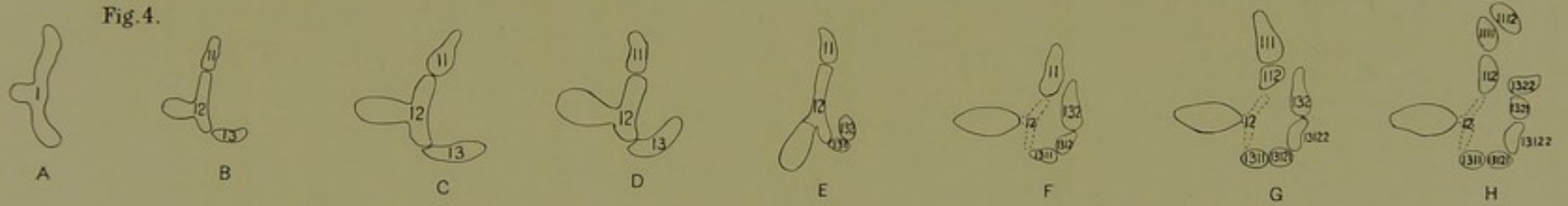


Fig. 5.

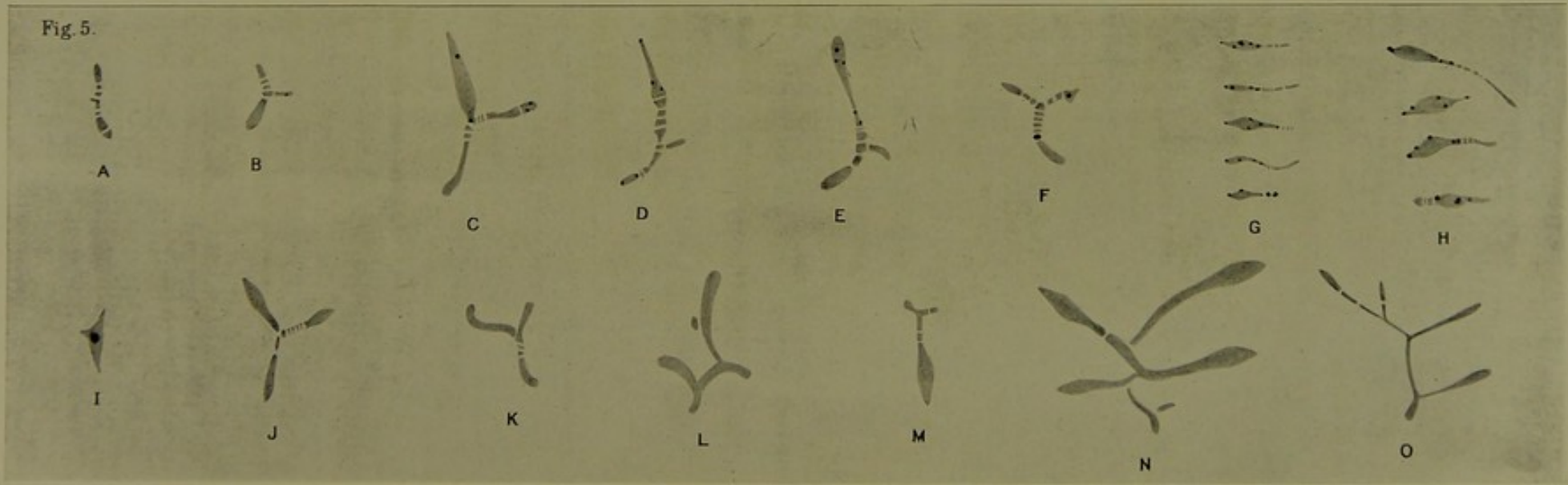


PLATE X

asiaticæ, as well as *B. xerosis* and *B. pseudodiphtheriæ*. They found snapping to occur only in the last two species and *B. diphtheriæ* and believe that snapping movements distinguish this group on the one hand from "typical bacilli" on the other.

Branching Diphtheria Bacilli.

Branching specimens of diphtheria bacilli have been met with in cultures and recorded by many observers, and Kanthack (12. IX. 1896) gives a short review of the observations made on this point up to 1896.

The principal hypotheses put forward in regard to branching are as follows:—

(1) That the branching is only apparent, and due to the accidental apposition of bacilli. The observations of Hill (1899, 1900 and 1902), Abbott and Gildersleeve (1903) and others have proved, however, that true branching does occur.

(2) That the bacillus pushes out a portion of its protoplasm which may constrict off or branch further without division (Pl. X, fig. 4). The researches of Hill (1902) prove that this form of branching occurs.

(3) That the bacilli are really made up of several individuals and that branching occurs when one grows out of the axial line. One of Hill's (1902, Pl. II, fig. 8) figures lends some support to this view.

(4) That branching is connected in some undefined way with the polar bodies[1]. (See Pl. X, fig. 5 C.) Babes (1895, Pl. X, fig. 3 A) pointed out that branches sometimes originate at points in the bacilli marked by polar bodies. Abbott and Gildersleeve (1903, p. 275), noticed that buds were often given off from points in the cells that were marked by the presence of irregular darkly stained masses, not the polar bodies.

(5) That branching is merely an exaggeration of the normal nodosities. Hill (1901) has shown that in many cases of branching this is the case.

(6) That branching is a degenerative change consequent upon growth under unfavourable environment.

As a result of his studies on hanging block and stained preparations Hill (1902) thinks that the following conclusions are justified in regard to branching:—

(1) Branching is not an accidental optical illusion.

[1] Branched forms with polar bodies are shown in Pl. X, fig. 5, A–F, and similar forms without polar bodies in fig. 5, I–O.

(2) Passive degenerative changes may give rise to slight irregular changes which simulate branches (Hill, 1902, Pl. II, fig. 7).

(3) As a part of the active development of the diphtheria bacillus, active branching by apparent budding, ending in the protrusion of an oval or elliptical body probably itself capable of further development and the production of new rods, may occur in young cultures, the parent stem then degenerating (Pl. X, fig. 4).

(4) As a part of the active development of the diphtheria bacillus, branching similar to that described, but terminating in an ordinary diphtheria rod-like body, without any degeneration of the parent stem at the point of origin, may occur on serum or agar within 17 hours, and this new rod may segment in the ordinary way or itself produce branches terminating in rods similar to itself, or in oval bodies such as above described (Pl. X, fig. 5, A—F, I—O).

(5) Various modifications of the processes described may exist.

The conditions under which branching occurs have been carefully observed by Abbott and Gildersleeve (1903). They find that it is not constant on any of the standard media, on which only a few of the organisms at any time show it. It is seldom observed on agar, potato, or broth. The presence or absence of oxygen does not affect it. They were unable to find any method which regularly ensures evidence of branching except in a very small proportion of the cells in a culture. Branching was most frequently obtained by these observers by cultivation at 37—38° C., on a solidified mixture of three parts of thoroughly beaten whole egg and one part of peptone broth containing 2 % of glucose. On this medium, when slightly alkaline, 10 strains of virulent diphtheria bacilli grew well, and in nine of these branching was fairly frequently seen. When the medium was made acid, branching was more frequently seen together with degeneration of the cells in the culture.

From the alkaline medium one branching form was met with in every three or four cover-glasses, whereas from the acid medium about every cover-glass contained one.

Hill (1900) investigated the frequency of branching forms in 648 positive cultures from cases of diphtheria and found them in 64 (10 %). He (1899) is convinced from his researches that at least 50 % of diphtheria cultures contain a few branching diphtheria bacilli.

Bernheim and Folger (1896) remark that they have occasionally encountered membranes rich in branching forms, but from these membranes they were unable to obtain colonies on serum.

As a result of their experiments Abbott and Gildersleeve agree with Migula (1900) and regard branching as evidence of degeneration and think the phenomenon of branching does not warrant diphtheria bacilli being classified with the hyphomycetes in which group they have been placed on this account by Lehmann and Neumann (1896).

With the view of determining if, in the course of development under the conditions found to be most favourable for branching, any evidence of a relation between the branched cells and true mycelial formation could be detected Abbott and Gildersleeve (1903) made various experiments. Cultures of the strains which were found to be most prone to branching were evenly distributed over solidified slightly acid egg albumen mixture in a Petri dish and incubated at 37° C. Impression preparations were made at the end of every two hours. They failed however to detect any suggestion of mycelial formation.

In this connection mention must be made of the following observations. Spirig (1899) states that if cultures of diphtheria bacilli be left for long periods colonies may rarely be found with a chalky appearance. Preparations from such colonies show not only club-shaped bacilli but thread-like and coccus-like forms, which are reproduced on subcultivation. These organisms are non-virulent. Concetti observed (1901) in a mild case of diphtheria organisms which possessed both diphtheria-like and long streptothrix-like forms. In neither instance can contamination with a slow-growing streptothrix be excluded.

Cache's (1901) experiments on this subject are more convincing than those which have just been quoted. A culture of the diphtheria bacillus made on Ouchinsky's mineral solution after some months growth showed a yellowish membrane, which consisted of ramified and interlaced mycelium including refractive granules. Subcultures showed similar growth, but transplantations on agar and gelatin after a few generations showed typical diphtheria bacilli capable of killing guinea-pigs within 40 hours, and of producing toxin.

Spores.

Diphtheria bacilli do not form spores under any known conditions, though at one time it was considered that the polar bodies might in some way be connected with spore formation.

Reaction to Staining Agents.

Diphtheria bacilli take up the basic aniline dyes with great readiness. The staining reagent generally employed for diagnostic work is *Loeffler's methylene blue* solution, which consists of

Saturated solution of methylene blue in alcohol	30 c.c.
Solution of potassium hydrate in distilled water (1—10,000)	100 c.c.

Fixed films may be stained by the application of this solution for

five minutes or longer in the cold. They are then washed in water, dried, and mounted in Canada balsam.

Stained by this means diphtheria bacilli show the irregularities of colouration already described, some showing marked segmentation, others a beaded appearance, while others are uniformly stained. In most cultures the majority of the diphtheria bacilli exhibit round or oval bodies clearly distinguishable owing to their dark violet-red colour. Not infrequently these bodies produce distinct swellings in the organisms, and sometimes one seems to occupy the whole of the dilated extremity of a bacillus. These bodies have been termed metachromatic granules, Babes Ernst corpuscles, or polar bodies, by various authors and are also known by several other names. They are usually situated near the ends of the bacilli and are generally surrounded by less darkly stained areas of protoplasm. One or more are, however, not infrequently to be found in the central portions. Throughout this section they are termed polar bodies. Special staining methods have been described, such as Neisser's method, for more clearly differentiating the polar bodies. Their relations to temperature, the age of the culture and its virulence are discussed under the description of Neisser's method.

The appearance of specimens stained by Loeffler's method are best appreciated by reference to Plates VII and VIII.

Cobbett and Phillips (1896) originally described a method by which films made from cultures may be stained and mounted in a dilute solution of Loeffler's methylene blue.

This method was very largely employed in his later work by Cobbett (1901) and the writer has found it to be extremely convenient, easy and reliable for diagnosis. The process consists in making on a cover-glass smears from the colonies on the culture medium. The top of a colony is touched with a platinum needle, and the point is then dipped into water and drawn across the cover-glass. The film thus made is allowed to dry and is then placed film side down on a glass slide on which has been placed a drop of dilute Loeffler's methylene blue (1 : 5). The specimen is almost immediately firmly pressed down on filter-paper with the cover-glass downwards, with the result that the excess of stain is forced from under the cover-glass and absorbed by the filter-paper. Immersion oil is then placed on the cover-glass, and the preparation thus mounted in a small quantity of the staining fluid is examined. If the films are properly prepared no bubbles are found under the cover-glass and the organisms take up the stain and appear to lie in a clear fluid. The segments or more obvious portions of protoplasm are stained

dark blue, and the presence of polar bodies is indicated by darkly stained, almost black, granules. (See also, p. 144, and Pl. VI.)

Gram's method. According to some authors diphtheria bacilli retain the stain by Gram's method and according to others they do not. This no doubt may be explained by the fact that the bacillus will not, as a rule, withstand a prolonged action of the decolourising fluid. Under the ordinary conditions of staining by Gram's method the bacillus certainly retains the dye.

Nicolle's method (1895), a modification of the above, has been extensively used by Woodhead (1896) and other observers. The staining fluid consists of 10 c.c. of a saturated solution of gentian violet added to 100 c.c. of a 1 % solution of carbolic acid. The fixed preparation is stained for five seconds and is passed directly into iodine solution (iodine 1 gram, potassium iodide 2 grams, distilled water 200 c.c.), where it remains five seconds. After this it is decolourised by being passed rapidly through a mixture of one volume of acetone with four volumes of absolute alcohol. This treatment removes all unfixed stain almost instantly. The specimen is then dehydrated in xylol and mounted in balsam.

The bacilli also stain well by the *Roux* and *Cladius methods.*

Special Staining Methods for differentiating the Polar Bodies.

The method most commonly used for differentiating the polar bodies is that introduced by *Neisser* (1896) in which two solutions are used.

No. 1.	Methylene blue (Grübler)	1 gram dissolved in alcohol (96 %) ... ... 20 c.c.
	Distilled water	950 c.c.
	Glacial acetic acid	50 c.c.
No. 2.	Bismark brown	2 grams.
	Boiling distilled water	1000 c.c.

The films are usually placed in solution No. 1 for one to three seconds, washed in water and placed for three to five seconds in No. 2, but considerably longer periods may be used with advantage especially with solution No. 2. The polar bodies of the bacilli are stained a dark blue, almost black, colour, and the protoplasm brown. Pl. XI, fig. 3 shows the characteristic features of a diphtheria preparation from a young serum culture stained by Neisser's method.

According to Denny (1903) polar granules are first seen as very minute objects after eight hours' growth, but do not become abundant

until the cultures have been growing about 15 hours. Neisser (1896) thought that they were characteristic of the diphtheria bacillus provided that cultures on Loeffler's serum were used and examined after 9 to 24 hours' incubation at 34—35° C. Even under these conditions, however, Hofmann's bacillus occasionally shows a few small and irregular polar bodies and some other organisms also exhibit them. The temperature at which the cultures are incubated has a marked influence on the appearance of the polar bodies. Even after 48 hours' growth at 19—21° C. they are very few (Denny, 1903). According to Neisser they are most frequently produced in cultures incubated between 34—36° C., but cultures growing at 37° C. also show them well. When cultures are incubated above this temperature the polar bodies may fail to appear (Neisser) or their appearance may only be delayed (Denny).

PLATE XI.

Fig. 1. Smear preparation from the throat in a case of diphtheria showing *B. diphtheriæ*, a few diplococci and cells. ×1000. (Photograph by Dr James Ritchie.)

Fig. 2. Groups of diphtheria bacilli in a section of membrane stained by Weigert's method. ×600 (Orig.).

Fig. 3. A preparation from a pure culture of *B. diphtheriæ* after 24 hours' incubation stained by Neisser's method. Zeiss $\frac{1}{12}$ Obj. Oc. 4. Drawn with the aid of a camera lucida. (Orig.).

Fig. 4. A preparation from the same culture as the last figure stained by Neisser's method after 48 hours' incubation to show the increase in the number and size of the dark staining areas. Zeiss $\frac{1}{12}$ Obj. Oc. 4. Drawn with the aid of a camera lucida. (Orig.)

In cultures 36 to 48 hours old many of the polar bodies are much enlarged and occupy a large part of the club-like dilations of the cells which have themselves become swollen. Some of these enlarged polar bodies are round, others oval and some elongated. Polar bodies only slightly enlarged and also others of the same size and shape as met with in younger cultures are also very common. In these old cultures the polar bodies appear to be increased in number as well as in size, so that several are often found in one bacillus.

Plate XI, fig. 4 shows the appearances seen in a film stained by Neisser's method from the same culture as Plate XI, fig. 3 after 24 hours' further growth.

Gossage (1896) made numerous experiments with media, to which glycerine had been added, and considered that the polar granules were

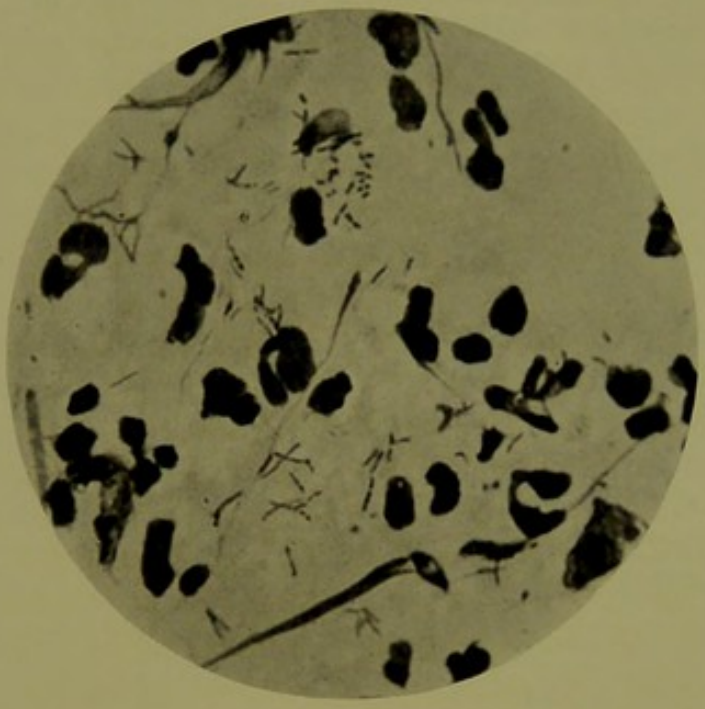


Fig. 1.

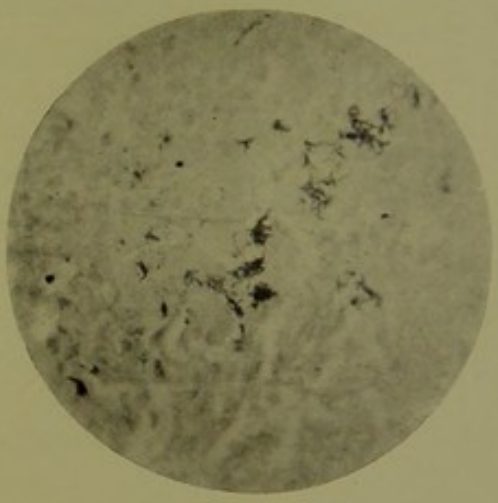


Fig. 2.

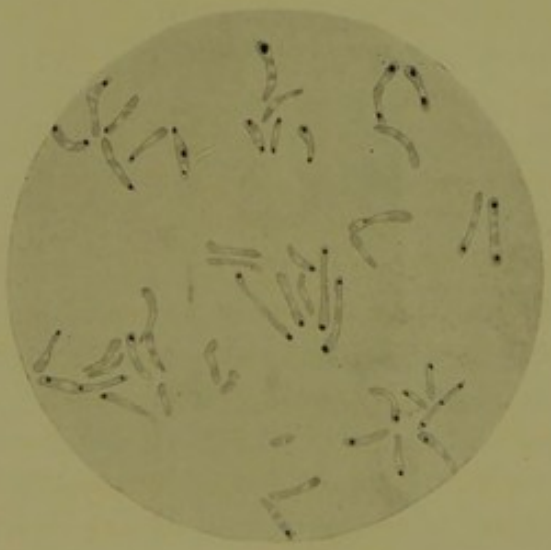


Fig. 3.

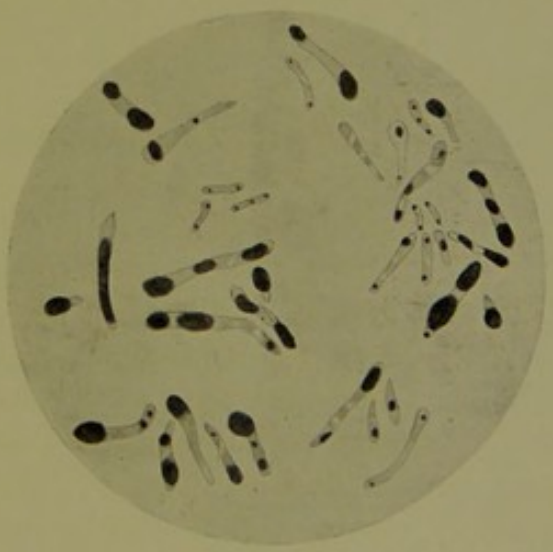


Fig. 4.

rendered more conspicuous by the addition of glycerine to the serum. Kanthack (22. VIII. 1896), however, denied that this was the case.

At one time it was frequently stated that the presence of polar bodies in diphtheria bacilli in cultures examined after 18—24 hours' growth at 36—37° C. indicated that the bacilli were virulent and that the absence of polar bodies indicated a lack of virulence. It has, however, since been proved by the observations of numerous workers that such is not the case. A certain proportion of diphtheria bacilli virulent for guinea-pigs show no polar bodies, and many non-virulent bacilli show well-marked polar bodies.

Reichenbach (1899), for example, found fully virulent bacilli without polar bodies and Park (1900) states that in his hands, with a very large experience, neither Neisser's stain nor its modifications have given any information as to the virulence of the bacilli. Of the 88 virulent diphtheria bacilli discovered by the writer (Graham-Smith, 1904, p. 277) in one epidemic 43·1 % showed well marked, 30·7 % small and poor, and 26·1 % no polar bodies; and of the 25 totally non-virulent diphtheria bacilli 88 % showed well marked, 8 % small, and 4 % no polar bodies. Schumberg (1902) concluded that there was no relation between the gravity of the disease in man and the presence of polar bodies.

Although Neisser's method is the one which has been most commonly used for differentiating the polar bodies, numerous other methods, some of them modifications of it, have been advocated for demonstrating their presence.

In all cases the films are, unless otherwise stated, fixed in the ordinary way and, after staining, washed in water, dried, and mounted in Canada balsam.

Crouch (1896) advocated the use of the following mixture:—

1 % Dahlia	1 part.
1 % Methyl green	5 parts.
Water	4 parts.

The films are placed in the mixture and almost instantly become sufficiently stained. The polar bodies are stained red.

Coles (1899) failed to obtain good results by Neisser's method but stated that constant results could be obtained in the following way:—

(1) The films are stained in Neisser's blue for 10—30 seconds. (2) They are then washed in water and placed in Gram's iodine for 30 seconds, (3) and finally again washed in water and placed in vesuvin (Bismark brown) for 10—30 seconds.

The bacilli are stained brown with blue granules.

Bronstein (1900) first stained with a dahlia solution consisting of dahlia 1 part, alcohol (95 %) 20 parts, and glacial acetic acid 50 parts, for 30 seconds to one minute. After washing with distilled water the films are counterstained for 30 seconds with Bismark brown (1 %). The bacilli are stained a brown colour and the polar bodies red. By this means he obtained 135 positive results out of 136 different specimens.

Cobbett (1901) showed that after mounting and examining films from cultures in dilute Loeffler's methylene blue (1 : 5) the bacilli can be decolourised by running a drop of 5 % acetic acid under the cover-glass. The polar bodies then show up as dark blue granules in the very pale blue bacilli. The writer has used this method in several hundred examinations and found it to be quick, reliable, and easy.

Piorkowski (1901) recommended staining the preparations for 30 seconds to one minute in aqueous methylene blue (warm), decolourising with 1 % acid alcohol, rinsing with water and counterstaining for five seconds with 1 % watery eosin. The protoplasm is stained dark red and the polar bodies very dark blue.

Schauffter (1902) stained films for one minute in the following mixture:

Loeffler's methylene blue	10 c.c.
Pyronin (Grübler) 5 grams dissolved in 10 c.c. of distilled water	1·5 c.c.
Hydrochloric acid (25 %) 3 c.c. in absolute alcohol 97 c.c.	·5 c.c.

The bacilli appear blue and the polar bodies red.

Falières (1902) made use of a stain consisting of

Methylene blue	2·0 grams.
Borax	·5 „
Distilled water	100·0 c.c.
Absolute alcohol	8 drops.

After staining the films are washed and counterstained with vesuvin for 30 seconds. The polar bodies stain very darkly and are sharply defined in the brown bacilli.

Ficker (1902) used methylene blue (Höchst) 1—10,000 to which 2 % of pure lactic acid had been added. He leaves the films in the stain for one minute. As in Cobbett's method the polar bodies are darkly stained and the rest of the bacillus remains almost unstained

De Rovaart (1902) stained the films for 1—1½ minutes in warm

Loeffler's methylene blue, washed in water and counterstained in vesuvin (1 %) for 1—1½ minutes. The bacilli are stained as in Neisser's method.

Peck (1903) stained the films for 3—4 minutes in Loeffler's methylene blue, washed quickly in water and counterstained in ·2 % vesuvin for 30 seconds. The bacilli are stained brown and the polar bodies blue.

Pitfield (1903) made use of three solutions :—

(*a*)	Silver nitrate	5 parts.
	Saturated alcoholic fuchsin	5 „
	Distilled water	3 „
(*b*)	Pyrogallic acid	1 part.
	10 % caustic soda	5 parts.
	Distilled water	10·0 „
(*c*)	Carbol fuchsin	10 drops.
	Distilled water	10 c.c.

The films are first stained in solution (*a*) which is kept warm, then washed and placed in solution (*b*), and finally stained in solution (*c*) for two minutes.

The bacilli are stained red and the polar bodies which take on a blackish colour are sharply defined.

Neisser (1903) has recently made use of a modification of his original method. His solution, No. 1, is retained and a second solution is substituted for the original No. 2.

Solution (2)	Crystal violet (Höchst)	1 c.c.
	Alcohol	10 c.c.
	Distilled water	300 c.c.

Solutions 1 and 2 are mixed in the proportion of two parts of the former to one of the latter, and films are stained in this mixture for one second, washed with chrysoidin (1·300 filtered) for three seconds, and then washed in water.

Andrade (1904) stains in a mixture of Borrel blue 1 c.c. and vesuvin (1 in 100,000) 50 c.c. for five seconds, washes with distilled water and places in Lugol's iodine solution for one minute, again washes in distilled water and decolourises with absolute alcohol till all the blue has come out, which will do so. The films are finally washed in distilled water, and mounted.

Pugh (1905) recommends the use of a mixture consisting of toluidine blue (Grübler) 1 gramme dissolved in 20 c.c. of absolute alcohol added to 1000 c.c. of distilled water and 20 c.c. of glacial acetic acid. The mixture is applied for two minutes. The protoplasm of the bacilli is stained a faint blue and the polar bodies by artificial light a reddish purple.

Higley (1905) has lately described a method for staining smears from membranes, which is only intended for the use of skilled laboratory workers when an immediate diagnosis is desirable. Two solutions are necessary which have to be freshly prepared.

No. 1 consists of five drops of Kühner's carbolic methylene blue in 7 c.c. of tap water, and No. 2 of 10 drops of carbol fuchsin in 7 c.c. of tap water.

No. 1 is applied for five seconds (not longer). The film is then washed, dried with filter-paper, and placed in solution No. 2 for 45 to 60 seconds and again washed. The diphtheria bacilli are stained a dark red or violet. He states that the bacilli are distinguished by their irregular staining, uneven contour, and the presence of blue polar bodies in some of them.

Ljubinsky (1905) made use of a solution consisting of

Pyoktannin (Merck.)	... ...	·25 grams.
Acetic acid (5 %)	... ...	100·0 c.c.

Films are stained for 30 seconds to one minute, washed and counter-stained with 1 % vesuvin for 30 seconds. The bacilli are stained violet, the polar bodies a very dark blue, and appear very large and distinct.

Epstein (1906) used the following method. He stains the fixed film in

1. Either 1 % pyronin solution or Loeffler's methylene blue for 30 seconds.
2. Rinses in tap water.
3. Treats with Gram's iodine for 10 seconds.
4. Rinses in tap water, and dries.

No counter stain is required. When pyronin is used the polar bodies are large, dark and brick red, and the bacilli light red. When Loeffler's blue is used the polar bodies are greenish black, and the bacilli greenish.

Loeffler (1907) has recently described a new method which he considers to be the best for demonstrating the polar bodies. Three solutions are used:

A. Aqueous solution of Borax (2·5 %) and methylene blue (1 %) 4 pts.
Unna's Polychrome methylene blue (Grübler) ..... 1 pt.
B. Eosine A. G. extra (Höchst) ·05 % aqueous solution
C. Tropaeolin O. O. aqueous solution..... 5 pts.
Acetic Acid ..... ·5 pt.
Water .....100 pts.

The film is stained for one minute in a mixture of equal quantities of A and B and then dipped in C and washed with water. The bacilli are pale and the polar bodies dark blue.

Blumenthal and Lipskerow (1905) have recently made numerous comparative experiments on preparations from cultures and membranes with the methods devised by Neisser, Crouch, Bronstein, Coles, Piorkowski, Pitfield, de Rovaart, Falières, Schauffter, Ficker, Peck, and Ljubinsky. They regard Falières' and Ljubinsky's methods as the most satisfactory, both on account of the sharp definition, size, and deep staining of the polar granules, and of the clearness with which the bodies of the bacilli are outlined.

Special Staining for Demonstrating the Capsules.

Boni (1900) recommends the following method for demonstrating the presence of capsules in diphtheria bacilli.

A fluid is prepared consisting of the white of one egg, 50 c.c. of glycerine, and two drops of formalin. The whole is shaken up and filtered. A small drop of this fluid is placed on a cover slip and mixed with diphtheria bacilli from a culture. The preparation is then allowed to dry and stained with Ziehl's carbol fuchsin for 20—30 seconds, rinsed with water, and dried. It is then stained with Loeffler's methylene blue for four to six minutes, rinsed with water, dried and mounted in balsam. The ground is then stained red, and the bacilli blue, with a clear capsule round them. Preparations made in this way certainly show the appearance described by Boni.

Feinberg (1900) stained diphtheria bacilli by Romanowski's stain and various modifications of it. The bacilli stained a bluish colour, and some showed within them two or three red dots, and others large red masses with a clear network in the middle. Feinberg seemed to regard these as nuclei.

The advantages and disadvantages of some of the most commonly

used stains in the bacteriological diagnosis of diphtheria will be discussed in Chapter X.

PLATE XII.

Fig. 1. Photograph of a culture of *B. diphtheriae* after 48 hours' growth on alkaline serum. (Orig.)

Fig. 2. Three serum cultures each inoculated with approximately $\frac{1}{100000}$ c.c. of mucus from the throat of a patient suffering from diphtheria. The cultures have been incubated for four days at 37° C. The raised well-defined colonies present in each case are colonies of *B. diphtheriae*. (From Gordon 1903, Pl. VI. fig. 1.)

Fig. 3. Colonies of *B. diphtheriae* after several days' growth on alkaline serum at 37° C. (Orig.)

Fig. 4. Colony of *B. diphtheriae* (type *a*) after 2 days' incubation at 37° C. on agar, highly magnified. (Orig.)

Fig. 5. An agar culture inoculated with a dilution of the nasal discharge of acute coryza and incubated for 4 days at 37° C. The large colonies are formed by a coccus and by Hofmann's bacillus. The minute colonies, in the majority, are colonies of *B. coryzae segmentosus*. (From Gordon, 1903, Pl. VI. fig. 2.)

The diphtheria bacillus is *non-motile and does not possess flagella.*

Cultivation on Artificial Media.

The methods of procuring pure cultures, the advantages and disadvantages of the various media and the differentiations of diphtheria-like organisms are given under the heading of "bacteriological diagnosis." The characters of the growths and the morphology of the bacilli in pure culture are alone dealt with in this section.

Solid media made from serum or serous exudates.

Owing to their selective action in favouring the rapid growth of diphtheria bacilli and retarding that of most other organisms, some of the media made from serum or serous exudates are first described.

Loeffler's serum.

The medium known as Loeffler's serum medium is that which has been most extensively used in the cultivation of diphtheria bacilli, and many observers unhesitatingly state that it is far superior to any other. The medium is made by adding three parts of ox, or horse, or other serum, to one part of neutral broth containing 1% of grape sugar, 1% of peptone and ·5% of salt. The whole is rendered slightly alkaline and the mixture is solidified in sloping tubes in a Koch's sterilizer, and subsequently sterilized in steam at about 90° C. The medium may also be first sterilized at a low temperature,

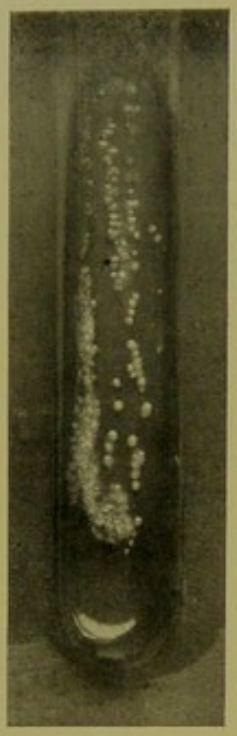


Fig. 1.

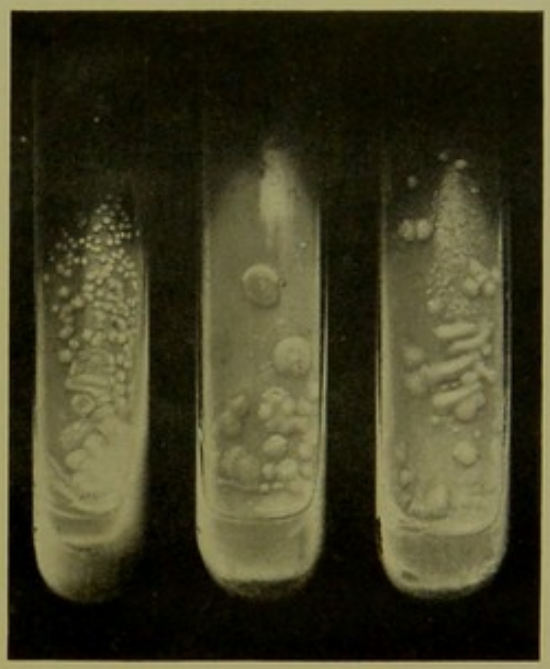


Fig. 2.

Fig. 3.

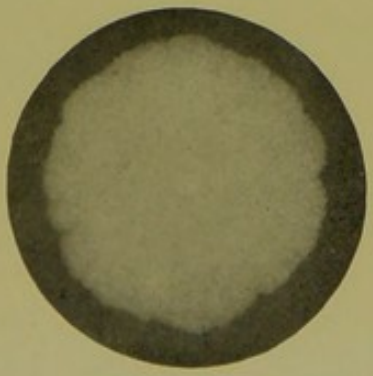


Fig. 4.

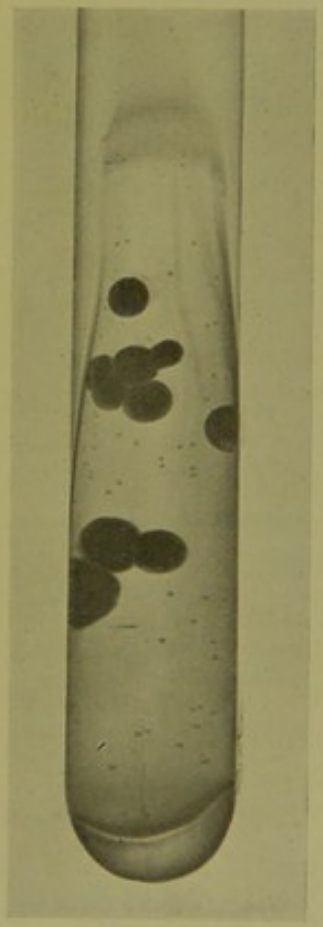


Fig. 5.

60—66° C. in steam on six consecutive days, and subsequently solidified. The resulting solid medium is of a whitish colour, opaque in the thicker parts, and gradually becoming clearer until it is almost transparent in the thinner parts of the slope. On this medium diphtheria bacilli grow very rapidly at temperatures between 35—37° C. and minute but distinct colonies may often be seen after 12 hours' growth. After 18—24 hours' incubation diphtheria bacilli produce rounded, elevated, moderately translucent, grayish-white colonies, with a slight yellowish tinge. This yellowish tinge, although very generally present, is slight. The colonies have a moist, shiny, smooth surface, and distinct or only slightly irregular margins. By transmitted light the centre owing to its greater thickness is opaque. When the colonies are few and separate they may grow to a considerable size in this time, but when numerous and close together they remain small. They are almost invariably discrete with distinct intervals between them.

In older cultures the colonies become more flattened, and their central opacity becomes more marked. They lose their moist shiny appearance and become dull, and the surface may become marked, especially round the opaque centre, by very slight irregular, concentric depressions or lines, indicating irregularities in the rate of growth. Not infrequently at this stage radial striation may also be seen, which may extend from the centre to the periphery. The margins no longer remain round but become slightly irregular, and finally crenated. The crenation of the margin is said to be due to the formation of fresh centres of rapid growth, which partially separate themselves from the original colony. This occasionally gives rise to an appearance as if a half, or a portion, of another colony was fused with the first (Pl. XII, fig. 2). Growth is very rapid for some hours after the colonies have become visible but gradually becomes less rapid after 36 hours. On somewhat old serum when the surface is dry, growth is impeded, and may not be rapid at any period.

In *stroke cultures* a gray streak is formed along the needle track. Subsequently growth takes place slowly in a lateral direction, but rapidly along the streak especially towards its lower end. Consequently a thickened raised band is formed down the centre thinning off laterally. The margins are sharply defined and distinctly crenated. At its upper end the streak thins off into isolated colonies with the characters described above. Owing to the fact that in this thinner portion the medium is always drier, these discrete colonies never attain large dimensions. Growth takes place between 22° C. and 40° C., but is most

pronounced between 35° C.—37° C. The morphology and staining reactions on this medium are those which have already been described.

Transparent Serum Media.

Lorrain Smith (1894) proposed a serum medium made by adding ·1—·15 % of caustic soda to ox serum and heating the mixture to 120° C. in the autoclave. By this means a transparent jelly was obtained. *Cobbett* (1898) used a modification of this medium, and later worked with a solidified serum medium made in the following way. To every 100 c.c. of ox or horse serum about 1 c.c. of a 10 % solution of caustic soda is added[1]. After thorough mixing 1 % of glucose is added and tubes containing about 5 c.c. of the fluid are solidified and sterilized in a sloping position in steam at a temperature of 87° C.[2] The resulting medium is of a slightly yellowish colour, depending on the amount of colouring matter present in the serum, and quite transparent. The colonies of the diphtheria bacillus on this medium are similar to those produced on Loeffler's serum, but in their early stages are translucent and not so gray. Their subsequent growth is similar, but the yellow tinge is often more marked. (Pl. XII, figs. 1 and 3.)

Kanthack and Stephens' serum agar. In 1896 these authors described a medium made in the following manner.

To every 100 c.c. of serous exudation (pleuritic or ascitic) derived from human subjects 2 c.c. of a 10 % solution of caustic potash is added and subsequently 1·5—2 % of agar (previously soaked in acidulated water and washed). The mixture is then boiled and filtered through a hot funnel and 4—5 % of glycerin is added to it. It is then poured into test tubes and sterilized. The authors say that 2 % of glucose may be added, but state that this does not appear to improve the medium. After sterilization the medium is firm and clear. In making this medium certain precautions are necessary. A small quantity of the

[1] The precise amount of caustic soda solution which it is necessary to add has to be determined by a trial, but according to the writer's experience of many thousand tubes of this medium 1 % is nearly always the quantity necessary. If too much soda is added the medium is very transparent but soft, and if too little, not so transparent, but very firm. The trial is conducted by taking about 5 c.c. of the serum with 1 % of soda solution added and heating in a small steam sterilizer in a sloping position. More serum or more alkali is added according to the result.

[2] Cobbett has shown that this temperature can easily be obtained by passing steam from an autoclave through a Bunsen burner into a closed box containing the tubes in a sloping position. When the requisite temperature has been reached, air is allowed to pass in with the steam by adjusting the collar of the burner to such an extent as to maintain a constant temperature.

exudate should be boiled in a test tube. If it becomes practically solid, owing to the presence of much albumen, it should be diluted to twice its bulk with distilled water. If this is not done the medium becomes gelatinous and useless. The morphology and cultural characters of the bacilli are much the same as on Loeffler's serum.

Hayward (1895) has suggested a medium made by catching hydrocele fluid in sterile flasks, decanting into test tubes, and sterilizing at 55° C. for three successive days. In the intervals it is kept at 37° C. The tubes are finally slanted and solidified in hot air at 68° C. The bacilli grow in white colonies and retain their usual morphological characters.

White (1895) also employed hydrocele fluid for the preparation of a transparent medium. He added ·07 % of a 10 % solution of caustic soda and sterilized and slanted in the autoclave at 120° C. for 10 minutes.

Horse or ox serum solidified in a Koch's sterilizer and subsequently sterilized in steam at 90° C., or first sterilized at a low temperature and later solidified, forms a medium on which diphtheria bacilli grow well with their usual characteristics.

Several other media containing serum, some of them with complicated methods, have been proposed, but have not been extensively used.

Nutrient agar. Nutrient agar forms a good medium for the diphtheria bacillus, but the growth is not so abundant as on Loeffler's serum. After 24 hours' incubation the colonies are usually still small. The colonies to be found on agar are of two types, though in their growth on other media and their pathogenic action on animals the strains of diphtheria bacilli which give rise to them are indistinguishable. One of these types (*a*) is small, gray or grayish white, almost translucent, rounded, and slightly raised, with a more or less granular surface and dark centre. Sometimes the margins are nearly circular, at other times slightly irregular, or more rarely markedly irregular shading off into a delicate lace-like fringe (Pl. XII, fig. 4). The other type (*b*) of colony, which is less common, is decidedly larger and more luxuriant. It is white, raised, and rounded, with a slightly granular or nearly smooth somewhat moist-looking surface. The culture has the same appearance as that shown on Pl. XII, fig. 1. Growth is often very feeble on agar when the bacilli are freshly obtained from the pseudo-membrane, but becomes more luxuriant after a few transplantations, when the organisms have become accustomed to it. In some cases even after several days' growth the colonies do not become much enlarged, but in others they grow to a considerable size and exhibit the characters of the colonies on

old serum cultures. In *stab* cultures growth occurs to the bottom of the line of inoculation as a gray line or as a series of small discrete colonies. Surface growth occurs either in the form of a central white mass with a thin surrounding expansion, or a white smooth dome-shaped mass. In stroke cultures a continuous layer of dull whitish colour is formed, the margins generally showing isolated colonies.

Morphology. In very young agar cultures the bacilli are usually somewhat shorter than on serum, but present otherwise the same morphological characters. In slightly older cultures they are not infrequently very long and very markedly segmented. Polar bodies are frequently absent. Involution forms become common sooner than on serum.

Glycerine agar. Glycerine agar has been very extensively used as a culture medium for diphtheria bacilli. Various proportions of glycerine between 2% and 8% have been added by various workers. Gossage (1896) observed the effects of glycerine[1] in glycerinated agar cultures made direct from the throat varying the percentage of glycerine between 6% and 18%. He found that as the proportion of glycerine increased the cultures became less impure, owing to the fact that the growth of the ordinary throat organisms was more impeded by the glycerine than that of the diphtheria bacillus. With 6% of glycerine added the growth was much the same as on serum. With high proportions of glycerine, however, the growth of the diphtheria bacilli was slow. The characters of the colonies on glycerine agar are much the same as upon nutrient agar, the majority being of type (*a*). The morphology of the bacilli on glycerine agar is the same as on nutrient agar.

Joos (1896) recommended a medium made of neutral peptone broth 1000 c.c., albuminate of soda 20 grams, and agar 20 grams, rendered alkaline by the addition of ·15% of normal caustic soda. He states that on this medium diphtheria bacilli produce good colonies in 15 hours.

Schloffer (1893) used a medium consisting of broth containing 2% agar 2 parts, and sterile urine 1 part. The bacilli are stated to be rather short and to show very few degeneration forms on this medium.

Capaldi (1896) considered egg agar to be an excellent diagnostic medium, colonies developing in 16 hours. Ordinary agar in tubes is melted and cooled to 45° C. and to each tube 3 or 4 loopfuls of sterile yolk of egg is added and mixed with the agar. The egg agar is allowed to set in a slope.

Gelatin. On gelatin the diphtheria bacillus grows at a temperature of 22° C.—24° C. or a little lower. Growth is slow and the gelatin is never liquefied. The appearance of most of the colonies is the same as that of the colonies on serum, and they undergo the same changes on

[1] For the effects of glycerine on the formation of polar bodies see page 142.

prolonged cultivation. The colonies soon become dull and lose their shiny appearance. Cobbett and Phillips (1896), who made very careful observations on the appearance of the colonies, describe them as follows: "The colonies of the virulent diphtheria bacilli were of two types, which for the sake of convenience may be termed large and small. The large colonies were for the most part of a whitish colour, with a raised centre and margin, separated by a circular depression. The outline was circular and showed little or no indentation, and only slight radial striation. More rarely the colonies were flatter and thinner, and were definitely divided by a number of radial fissures so that they exactly resembled the daisy-shaped colonies which have been described as occurring on serum. The small colonies were extremely minute. With a lens they were seen to be thin and of the daisy shape." Both kinds may occur in one culture. In *stab* cultures a line of small colonies is formed along the needle track, whilst on the surface a small whitish disc is produced somewhat thicker at the centre.

Morphology. The bacilli show the same morphological and staining characters as those from serum cultures, except that the polar bodies are less prominent and fewer in number.

Potato. Many of the earlier writers state that growth does not occur on potato. On this medium however, especially when the surface is made slightly alkaline, a fairly abundant growth occurs, but can only be seen with difficulty. Growth also occurs on potato steamed without any preliminary treatment. Frequently it is entirely invisible, or may be only indicated by a dry, thin, glaze after some days' incubation. More rarely a perceptible whitish growth occurs, or one with a very slightly yellowish tinge. This character of almost invisible growth separates the diphtheria bacillus from several other bacilli morphologically resembling it.

Microscopical examination after 24 hours' incubation at 37° C. reveals a tolerably abundant growth even when none is perceptible on the surface. Irregular enlarged forms are particularly numerous in potato cultures, and in general the rods are thicker than on other media.

Alkaline bouillon (broth). Ordinary alkaline broth is a good and distinctive medium for the growth of diphtheria bacilli. Usually small whitish granules are formed at the bottom and along the sides of the tube and the medium remains clear without any cloudiness. Rarely a more or less diffuse cloudiness occurs, but the particles generally settle to the bottom in 48 hours or less. Sometimes a slight film forms on the surface of the broth. On shaking the tube this film breaks up and slowly

sinks to the bottom. A film or membrane is more apt to develop on the surface of cultures which have been grown for several generations in broth. According to Theobald Smith (1896) the presence of a membrane seems linked to an acidity less than 1·5 % to phenol-phthalein, i.e. an acidity which is neutral or alkaline to litmus. He states that a membrane is, according to his observations, almost proof positive that the acidity is below a certain limit. When only traces of muscle sugar are present a vigorous surface growth begins at once. He is inclined to attribute this to the reaction of the fluid and its action on the bacilli, which he explains from the following observations. According to Chittenden and Gies (*Journ. of Exp. Med.* 1896, I. p. 186) mucin in acid solution sinks to the bottom, but when the fluid is neutralized the mucin swells and rises to the top. The outer envelope of most bacteria, according to Welch, appears to be composed of a substance which behaves like mucin during the process of staining.

Morphology. In the earlier stages of their growth on broth the bacilli are usually slender and less segmented than on other media. But even at an early period irregular and enlarged forms are common, and typical involution forms make their appearance after 3 or 4 days' growth.

The products of the growth of the diphtheria bacillus in broth. In its growth the diphtheria bacillus splits the meat sugar contained in ordinary broth producing acid and thus changes the reaction of the medium, rendering it slightly less alkaline, or even slightly acid. After a variable time the reaction frequently again becomes alkaline.

Roux and Yersin (1888, p. 629) were the first to observe these changes in reaction, and their observations have been confirmed by all subsequent investigators.

Theobald Smith (1893, *Wilder Quarter-century Bk.* p. 224) first pointed out that if diphtheria bacilli be cultivated in broth free from muscle sugar or nearly so, the reaction remains alkaline from the start. Two years later Spronck (1895) independently came to the conclusion that when diphtheria bacilli are grown in broth free from muscle sugar the reaction remains alkaline, when small quantities of sugar are present the reaction becomes acid and later alkaline, but when much sugar is present the broth becomes acid and remains permanently acid. Since that time numerous observers have confirmed the results of these experiments, and have more fully investigated the effects of these changes.

Theobald Smith (1897) tested numerous samples of broth made from beef, and found that the quantities of muscle sugar varied in

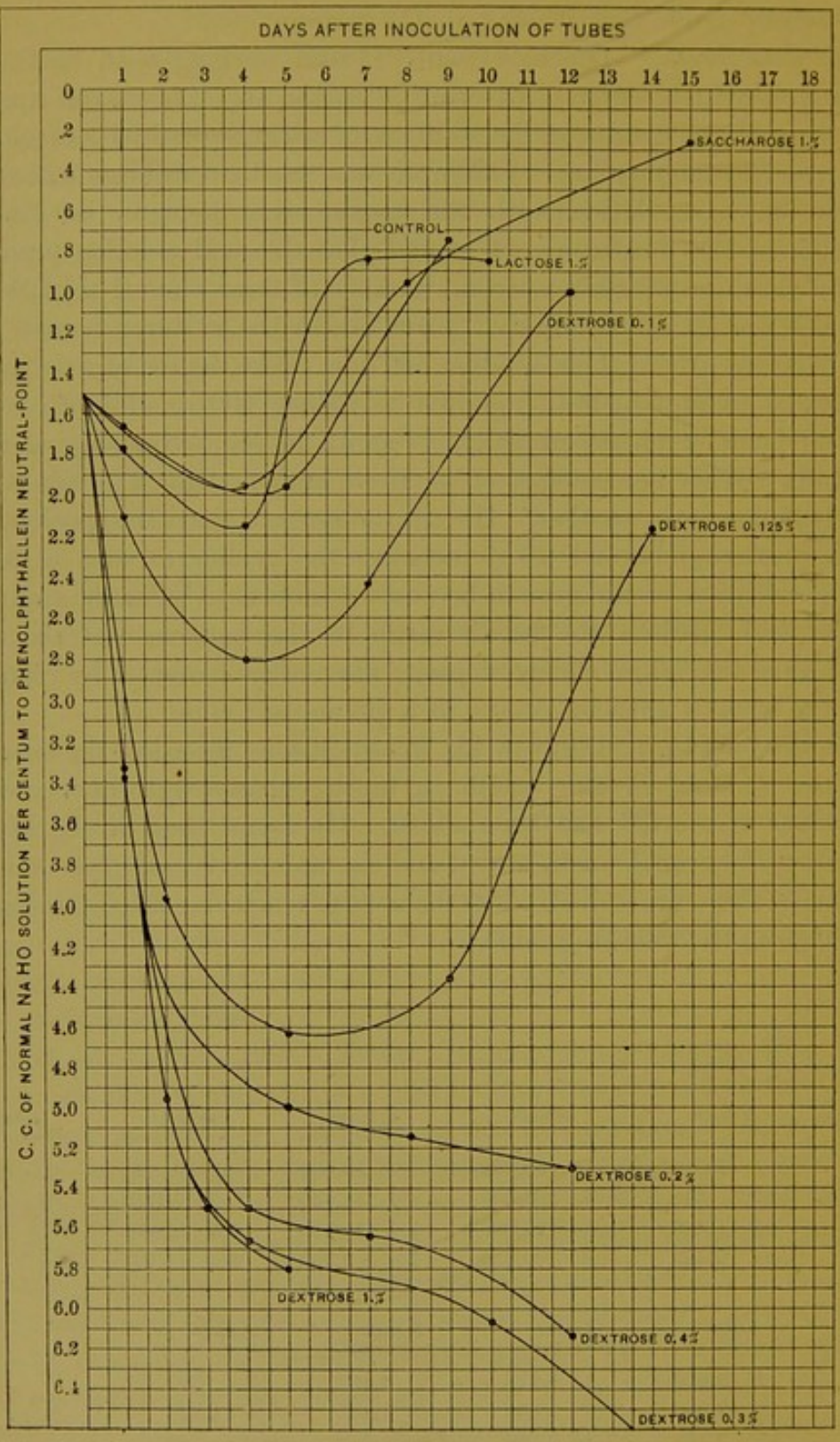
different samples from the same places at different times of the year. He usually found less present in the winter.

Glucose broth. Theobald Smith (1896) working with broth containing very little sugar, as determined by the action of *B. coli* upon it, investigated the results of the addition of various quantities of glucose (dextrose) to the medium. The broth was sterilized in large test-tubes, which after inoculation with the bacilli were kept in an inclined position in the incubator. He found that the acid reaction promptly formed disappeared after a certain number of days when ·1 to ·125 % of dextrose was used, but when ·2 % was added the cultures became acid and never recovered their alkaline reaction. The degree of acid in c.cs. of normal sodium hydrate required to reduce 100 c.c. of broth to the neutral point of phenol-phthalein is shown in the accompanying chart (p. 156). With percentages of dextrose higher than ·2 % the curves closely follow one another. He also observed that the presence of sugar (dextrose) beyond ·15 % increases the acidity to such a degree that a cessation of the growth becomes manifest. Under the influence of the acid medium the bacilli settle to the bottom, coat the glass with a peculiar thin cloudy deposit, not easily removed by shaking, and the growth thereby comes to a premature close.

Hellström (14. II. 1899) in his investigations recorded the reaction and the number of the bacilli in broth cultures to which varying quantities of glucose had been added. He found that as the acidity increased the growth was retarded and the number became less and less, till finally the culture died, when the acidity reached − 2·5. His figures are given in the following table :—

% of glucose in ordinary broth of initial reaction +·2	20 hours		30 hours		42 hours		60 hours		4 days	
	Reaction	Bacilli per loop								
0 %	+·1	1,020	−·15	29,700	− ·2	10,800	− ·1	976	− ·15	2,984
·1 %	+·1	521	−·4	86,400	− ·7	14,850	− ·9	2,700	− ·9	1,840
·2 %	+·1	1,396	−·6	78,300	−1·4	12,150	−1·6	3,860	−1·7	4,050
·3 %	+·1	2,432	− ·8	60,750	−1·9	5,400	−2·5	45	−2·5	61

	5 days		7 days		10 days		21 days	
0 %	− ·2	3,670	− ·2	1,215	− ·2	4,050	− ·2	992
·1 %	− ·9	2,950	− ·9	2,600	− ·9	1,245	−1·1	1,528
·2 %	−1·75	3,120	−1·85	3,340	−1·9	808	−2·0	148
·3 %	−2·45	0	−2·5	0	−2·5	0	−2·5	0


DAYS AFTER INOCULATION OF TUBES
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
C. C. OF NORMAL NA HO SOLUTION PER CENTUM TO PHENOLPHTHALLEIN NEUTRAL-POINT
0 .2 .4 .6 .8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 3.6 3.8 4.0 4.2 4.4 4.6 4.8 5.0 5.2 5.4 5.6 5.8 6.0 6.2 6.4
SACCHAROSE 1.%
CONTROL
LACTOSE 1.%
DEXTROSE 0.1%
DEXTROSE 0.125%
DEXTROSE 0.2%
DEXTROSE 1.%
DEXTROSE 0.4%
DEXTROSE 0.3%

From his figures it is seen that in ordinary broth, to which no glucose had been added, but little change in reaction occurred, and the bacilli increased in numbers up to 30 hours and subsequently diminished, until after 21 days' growth their number was about the same as at the start. The addition of ·1 % of glucose caused a gradual increase in acidity. The number of bacilli increased up to 30 hours and then gradually diminished. The addition of ·3 % caused a rapid increase in acid and the death of the culture in five days.

Cobbett (1897) confirmed the general conclusions of these observers, but states that contrary to the case of *B. coli* and other bacteria the diphtheria bacillus is not destroyed by the formation of acid in glucose broth, for cultures can be obtained after months.

Almost all workers who have investigated the changes of reaction in broth due to the growth of diphtheria bacilli have used either litmus or phenol-phthalein as indicators. Bronstein and Grünblatt (1902) have recently recommended another method. Their reagent consists of

(1) 2 c.c. of indigocarmine in 100 c.c. of distilled water.

(2) 10 c.c. of acid fuchsin in 100 c.c. of 1 % caustic soda.

Before use two parts of No. 1 are added to one part of No. 2 and 22 parts of water. Some of this mixture is added to the broth before it is placed in test-tubes. Control tubes remain blue, but tubes containing diphtheria bacilli turn a ruby-red colour.

The Action of the Diphtheria Bacillus on Various Sugars and Carbo-hydrates.

Although all investigators are agreed that the diphtheria bacillus acts powerfully on glucose and produces acid, the few observations which have been made on its action on other sugars and carbo-hydrates are not all in agreement. Although little attention has been paid to it, this subject is one which may turn out to be of considerable importance in aiding in the differentiation of the various diphtheria-like organisms from one another and from the diphtheria bacillus.

L. Martin (1898) states that the diphtheria bacillus produces acid from glucose, galactose, laevulose, glycerine and saccharose, but not from lactose, maltose, raffinose, arabinose, dulcite or mannite. The careful experiments of Theobald Smith (1896), however, show that neither lactose nor saccharose is acted on, and most other writers, who have mentioned the subject, agree that acid is not produced from saccharose. Blumenthal disagrees with both these writers in stating

that lactose is split up by the diphtheria bacillus. More recently Knapp (1904), working with the serum-water medium of Hiss, has investigated the acid-forming power of 27 races of diphtheria bacilli. He finds that when glucose, maltose, dextrine or mannite are present, the medium is coagulated and made acid, but that lactose and saccharose are not acted on. Hamilton and Horton (1906) found that all races of typical diphtheria bacilli produce acid in media to which dextrine has been added. The writer (Graham-Smith, 1906) has recently investigated the action of 23 races of typical diphtheria bacilli, both virulent and non-virulent, obtained from the throats of persons suffering from diphtheria, convalescents, and contacts, on 1 % solutions of glucose, galactose, laevulose, maltose, dextrine, glycerine, lactose, mannite, and saccharose in the serum-water medium of Hiss and in sugar-free broth. Neutral litmus was used as the indicator. Two sets of experiments were made with Hiss's medium, one series being grown at 37° C. for three days, the other for ten days. The broth cultures were incubated for 48 hours. Acid was never produced in media containing saccharose or mannite. An acid reaction was invariably produced in media containing glucose, galactose, and laevulose. In Hiss's medium (10 days' culture) every specimen produced acid with maltose, and all but one with glycerine, and all but two with dextrine. With lactose six showed a marked acid reaction, ten a slight acid reaction, and four no acid reaction, while in three the reaction was doubtful. Marked coagulation of the medium occurred in almost every tube containing glucose, galactose, laevulose, maltose, and dextrine, and in half the tubes containing glycerine and lactose. In the latter two media many of the tubes were only partly coagulated and in some the medium was only made more viscid or gelatinous. No change was noticed in tubes containing saccharose or mannite. In the series incubated for three days the acid reaction failed to appear in many of the tubes containing lactose and glycerine, and in some containing dextrine and maltose. In the broth cultures acid was always produced with glucose, galactose and dextrine, and sometimes with maltose, lactose and glycerine.

The difference between the series grown on broth and on Hiss's medium is probably due to the fact that some of the bacilli grew poorly on the former, but all grew well on the latter. This corresponds with the well-known facts that recently isolated diphtheria bacilli grow well on media containing serum, but often grow poorly at first in broth. In broth, therefore, the reaction only took place in the presence of those substances on which diphtheria bacilli act readily.

Table showing the action of diphtheria bacilli from different sources on various sugars and carbo-hydrates in the serum-water medium of Hiss and in broth.

	Source	Virulence	Hiss's medium (10 days' growth)									Broth (48 hours' growth)							
			Glucose	Galactose	Laevulose	Maltose	Dextrine	Glycerine	Lactose	Mannite	Saccharose	Glucose	Galactose	Maltose	Dextrine	Glycerine	Lactose	Mannite	Saccharose
1.	Clinical case	virulent	C	C	C	C	C	C	C	–	–	+							
			+	+	+	+	+	+	+	o	o								
2.	Convalescent (26th day)	,,	C	C	·	C	C	c	C	–	–	+	+	o	*	o	o	o	o
			+	+	·	+	+	+	+	o	o								
3.	,, (10th day)	,,	C	C	C	C	C	C	c	–	–	+							
			+	+	+	+	+	+	*	o	o								
4.	Contact	,,	C	C	C	C	C	C	C	–	–	+							
			+	+	+	+	+	+	*	o	o								
5.	,,	,,	C	C	C	C	C	C	C	–	–	+							
			+	+	+	+	+	+	*	o	o								
6.	,,	non-virulent	C	C	C	C	C	C	c	–	–	+							
			+	+	+	+	+	+	*	o	o								
7.	Clinical case	virulent	C	C	C	C	C	c	C	–	–	+	+	o	+	o	o	o	o
			+	+	+	+	+	*	+	o	o								
8.	,, ,,	,,	C	C	C	C	C	c	C	–	–	+	+	o	+	o	o	o	o
			+	+	+	+	+	*	+	o	o								
9.	,, ,,	,,	C	C	C	C	C	c	C	–	–	+	+	+	+	o	*	o	o
			+	+	+	+	+	*	+	o	o								
10.	Contact	non-virulent	C	C	C	C	c	C	c	–	–	+							
			+	+	+	+	+	+	*	o	o								
11.	Convalescent (56th day)	virulent	C	C	C	C	c	C	c	–	–	+							
			+	+	+	+	*	+	*	o	o								
12.	Contact	,,	C	C	·	C	C	c	c	–	–	+	+	*	+	o	o	o	o
			+	+	·	+	+	*	*	o	o								
13.	Clinical case	,,	C	C	C	c	c	C	C	–	–	+	+	*	*	*	o	o	o
			+	+	+	+	*	+	*	o	o								
14.	Contact	,,	C	C	C	C	C	c	c	–	–	+	+	+	*	o	o	o	o
			+	+	+	+	+	*	*	o	o								
15.	Clinical case	,,	C	C	·	C	C	–	C	–	–	+							
			+	+	·	+	+	*	+	o	o								
16.	Contact	non-virulent	C	C	·	C	C	–	–	–	–	+	+	o	+	*	*	o	o
			+	+	·	+	+	*	o	o	o								
17.	,,	,,	C	C	·	C	C	c	–	–	–	+	+	*	+	o	o	o	o
			+	+	·	+	+	*	*	o	o								
18.	Convalescent (35th day)	virulent	C	C	C	c	c	C	g	–	–	+	+	o	+	o	o	o	o
			+	+	+	+	*	+	n	o	o								
19.	Contact	,,	C	C	C	C	c	C	g	–	–	+	*	o	*	o	o	o	o
			+	+	+	+	n	+	n	o	o								
20.	Convalescent (46th day)	,,	C	C	C	C	c	C	–	–	–	+							
			+	+	+	+	+	+	n	o	o								
21.	Clinical case	,,	C	C	C	c	C	c	–	–	–	+							
			+	+	+	+	*	*	o	o	o								
22.	Contact	,,	C	C	C	C	C	C	–	–	–	+							
			+	+	+	+	*	*	o	o	o								
23.	"Sore throat" ("sheath form")	non-virulent	C	g	C	C	–	–	–	–	–	+							
			+	*	+	+	o	o	o	o	o								

C = complete coagulation.
c = partial ,,
g = medium rendered of a gelatinous consistency.

– = no change in appearance.
+ = markedly acid.
* = acid.

n = slightly acid or neutral.
o = no acid formation.
· = not tested.

These tests show that the diphtheria bacillus acts on glucose, galactose, laevulose, maltose, and dextrine, producing an acid reaction, and generally on glycerine and lactose if incubation is continued for 10 days. Mannite and saccharose are not usually acted upon.

More recently the writer has had the opportunity of testing a number of bacilli obtained from outbreaks in several villages in each of which there was probably a single source of infection. Under these conditions it seemed possible that the bacilli isolated from different individuals in the same locality might exhibit similar powers of acting on the various substances mentioned in the serum medium of Hiss. Sixteen strains were isolated from five small outbreaks and compared with 22 bacilli from a larger outbreak. The change produced in the medium in each case was noted daily, and it was found that the degree and rate of coagulation varied as well as the apparent rate of acid formation. In some cases complete coagulation and a high degree of acidity were produced within 24 hours, while in other cases the same result was only reached after several days. All produced a marked acid reaction in the presence of glucose, galactose and maltose, and with one exception (23, slight reaction) in the presence of laevulose. With glycerine 31 produced a marked acid reaction, five (4, 5, 6, 15, 16) a feeble reaction and two (8, 9) no reaction. In no instance was any trace of acid produced with

	Strain	Lactose	Dextrine	Saccharose
Outbreak A	1	0	0	0
	2	0	0	0
,, B	3	0	*	0
	4	0	*	0
	5	0	*	0
	6	0	+	0
,, C	7	0	+	0
	8	0	*	0
	9	0	*	0
	10	0	+	0
,, D	11	+	+	0
	12	+	+	0
	13	+	+	0
,, E	14	·	+	0
	15	+	+	0
	16	+	+	0
Outbreak F	17	0	+	0
	18	+	+	0
	19	*	+	0
	20	0	+	+
	21	0	+	+
	22	0	+	+
	23	0	+	+
	24	0	+	+
	25	0	+	+
	26	*	+	+
	27	*	+	+
	28	*	+	+
	29	*	+	+
	30	*	+	+
	31	+	+	+
	32	+	+	+
	33	+	+	+
	34	+	+	+
	35	+	+	+
	36	+	+	+
	37	+	+	+
	38	+	+	+

mannite. Great differences were noted in the reactions on lactose, dextrine and saccharose, which are given in the preceding table.

The main point of interest is that bacilli from the same locality, and therefore probably derived from one source, usually behaved in the same way. None of those from outbreaks A—E produced any reaction with saccharose, but contrary to previous experience (see p. 159) the majority of strains from F produced a marked acid reaction.

These experiments prove that under suitable conditions all strains of diphtheria bacilli produce acid from glucose, galactose, laevulose, and maltose and the majority from dextrine and glycerine. The action on lactose is very variable and only a few strains act on saccharose. All tests on mannite yielded negative results.

For the action of Hofmann's bacillus and certain diphtheria-like bacilli on these substances see p. 204.

The Effects of Alkalis and Acids on the Growth of the Diphtheria Bacillus in Broth.

(*a*) *The effects of Alkalis.* Cobbett (1897) made a series of sugar free broth tubes (neutral to phenol-phthalein) each holding 5 c.c., containing 2% of peptone and ·5% of salt, and added sodium hydrate in quantities corresponding to 10, 20, 30, 40, 50, and 60 c.c. of normal alkali per litre. In tubes containing as much as 30 c.c. of normal alkali per litre the growth was good, in those containing 40 c.c. per litre it was retarded, but it was entirely inhibited by higher degrees of alkalinity. In another series the sodium hydrate was replaced by the alkaline products of the bacilli themselves obtained by distillation. The results were exactly the same.

(*b*) *The effects of Acids.* (Cobbett, 1897.) Three series of similar tubes were made, but containing acid instead of alkali, corresponding to 2·5, 5, 7·5, 10, 12·5, 15, 17·5, and 20 c.c. of acid per litre. In series A the acidity was produced by sulphuric acid, in B by hydrochloric acid, and in C by the products of an old diphtheria culture in glucose broth. The results of these experiments are shown in the following table:

Acidity corresponding to c.c. of acid per litre	Series A		Series B		Series C
	Set 1	Set 2	Set 1	Set 2	
2·5	growth	growth	growth	growth	growth
5·0	,,	,,	,,	,,	,,
7·5	no growth	growth retarded	growth retarded	growth retarded	growth retarded
10·0	,,	trace of growth	,,	,,	,,
12·5	,,	no growth	no growth	,,	no growth
17·5	,,	,,	,,	no growth	,,
20·0	,,	,,	,,	,,	,,

In all cases the growth was retarded by an acidity corresponding to 7·5 c.c. of acid per litre, and it was completely inhibited, except in one instance, by an acidity corresponding to 12·5 c.c.

The Influence of Sugar on Toxin Production.

Among the products formed by the growth of the diphtheria bacillus in broth are toxins, etc. These will be fully dealt with later (Section V), but some observations on this point must be mentioned here. The formation of acid from the muscle sugar in ordinary broth is not only of importance in diagnosing the diphtheria bacillus from other organisms, but has a considerable influence on the production of toxin.

Roux and Yersin (1888, p. 629) were the first to point out that the toxicity of the cultures begins with the alkaline reaction. Theobald Smith (1893) found that the amount of toxin was to some extent inversely proportional to the quantity of the muscle sugar in the broth. Spronck (1895) quotes an experiment which clearly demonstrates the influence of muscle sugar on toxin production. He experimented with two cultures, which in doses of ·2 c.c. after 24 hours' growth, injected subcutaneously, killed guinea-pigs of 250—300 grs. in 24 hours. These were sown on a series of broths made from meat obtained from different sources and incubated at 37° C. He found that the results might be divided into three groups. Group A:—The cultures became acid, and as the acidity increased the development was hindered and the filtrate was not toxic. Group B:—The cultures were luxuriant and the reaction alkaline. After three weeks' growth ·1 c.c. of filtrate killed 500 grm. guinea-pigs in 48 hours. Group C:—Cultures first became acid and later alkaline, but did not produce as much toxin as Group B. These differences he found depended on the amount of sugar in the meat from which the broth was prepared.

These conclusions have been amply confirmed by later investigators and are of great importance in the manufacture of toxins, but are here mentioned on account of their bearing on the pathogenic as opposed to the toxin producing powers of the bacillus.

Sugar-free broth.

From the observations which have just been recorded the necessity for obtaining a broth free from muscle sugar for testing the pathogenic and toxin producing powers becomes apparent. (Cp. section on virulence, p. 173.)

To get rid of the muscle sugar for the preparation of small quantities of broth several means have been adopted, of which the following are the best known:—(1) By allowing the meat to become stale or putrefy before making the broth, thereby making use of the putrefactive organisms to destroy the muscle sugar (Spronck). (2) By adding calcium carbonate to the broth to neutralize the acid as it is formed (Spronck). (3) By inoculating the meat extract with an acid-forming organism, and then after four or five days' proceeding to neutralize, filter, add peptone, and resterilize (Smith). Of these methods Nos. 1 and 3 have been most frequently used (see also Section V).

In testing a sample of broth for the presence or absence of sugar the following method devised by Theobald Smith (1896) is simple and reliable.

Fermentation tubes are filled with the broth, sterilized, and inoculated with *B. coli* or other acid producing bacterium. A simple inspection of the tube from day to day gives sufficient information of the muscle sugar present. If the closed branch of the fermentation tube remains clear, sugar is entirely absent. If it becomes clouded the cloudiness increases in density with the quantity of sugar present. If a little gas appears the quantity of sugar may be still larger in proportion to the gas accumulated. In all cases the open bulb must become clouded or turbid, otherwise the broth is unfit for the multiplication of bacteria.

Ascites broth.

Ascites broth, namely broth to which 25% of ascitic fluid has been added, forms a good culture medium for diphtheria bacilli. (See virulence experiments, p. 175.)

Nicolas and Arloing (1899) compared the growth of diphtheria bacilli on various kinds of broth: (1) ordinary broth, (2) broth made from putrefied meat, (3) ordinary broth to which 10% of human serum had been added, and (4) ordinary broth to which 10% of horse serum had been added. They found that growth was most luxuriant in the order named.

Media made from Various Organs.

Livingwood (1898) made media from the liver, spleen, and suprarenal bodies to determine whether the extracts exerted any marked influence. He found that very little difference was noticeable in the growths, except that the growths on media made from spleen and suprarenal extracts, filtered through porcelain and

not heated, were less than those on media made from these organs in the ordinary way.

Cesaris Demel (1899) grew the bacilli on liver broth to which neutral litmus had been added until a violet-amethyst colour was produced. In 24 hours the diphtheria bacillus gave a rosy colour and red deposit, but the Hofmann's bacillus made the broth violet, while the deposit was colourless.

Mayer (1899) made broth from the salivary glands of freshly killed animals (calf, ox, horse, and pig), and media from muscle-juice, urine, gall, aqueous humour, and mucin. He found that on these media slight differences of growth took place, but they were not of great importance.

Glaessner (1900) made numerous experiments to test the efficacy of various substances as substitutes for peptone. The substances used were asparagin, somatose, and nutrose, with and without glycerine, and these were tested against broth made with peptone either alone or combined with glycerine. The bacilli were counted at the start of the experiment, and after 11 and 15 hours' growth. A greater multiplication took place in the tubes without peptone than in those with it.

Milk.

In *milk* prepared in the usual way the diphtheria bacillus grows well, beginning to develop at a comparatively low temperature (20° C.). The milk remains unchanged in appearance and no coagulation occurs, but the medium becomes acid. (Cp. milk as a food substance, p. 172.)

The bacilli multiply in *Litmus whey* at 37° C. and turn the medium acid. This medium has been used instead of glucose broth to ascertain the acid forming power.

Egg media.

Fraenkel (15. IV. 95, p. 349) was the first to recommend cooked egg as a culture medium for the diphtheria bacillus.

"In *cooked and raw eggs* the diphtheria bacilli develop both in the white and the yolk very well; also on *solid egg-albumen* where it sometimes shows branched forms" (Bowhill, 1899, p. 164).

Indol.

It is stated by some writers that the diphtheria bacillus produces indol, while by others this is denied. These differences of opinion probably depend on the methods of testing. Gorini (1893) observed that cholera spirilla failed to give the cholera red reaction when ·5% of dextrose, saccharose, or lactose was present, and Kruse (1894) says that if the broth contains ·25% of sugar the indol reaction no longer appears. Theobald Smith thinks that peptone solution is a bad medium for testing the formation of indol, and considers dextrose-free broth to be better. He found that while in the former the reaction

only appeared in 3—6 days, in the latter it appeared in 16 hours, and was very bright. He also observed that in broth containing muscle sugar the reaction was only positive when the sugar had been converted into acid and the latter nearly neutralised. His experiments led him to conclude that the diphtheria bacillus formed no indol. Dzierzgowski and Rekowski (1895) grew diphtheria bacilli in flasks containing 2% peptone in distilled water for six weeks at 36·5° C., and kept control flasks under the same conditions. The distillate showed no free volatile or aromatic acids, or indol, skatol, tyrosin, or cadaverin. Petri (1890) and Kitasato (1889) could not find indol in 24—48 hour cultures, and Lewandowski (1890) was unable to detect it by distilling 8—10 day old cultures and testing the filtrate.

On the other hand Palmirski and Orlowski (1895) studied the filtrates from broth cultures, and state that the indol reaction appears after prolonged cultivation. Peters (quoted by Flügge), Hewlett and Knight (1897), and Hewlett (1900) agree with these observers in thinking that indol is produced after three or four weeks' growth. On further observation, Hewlett (1901) found that the quick reaction which he obtained by the addition of a strong acid and a weak nitrite solution to diphtheria cultures, was not due to indol, which is volatile, but to a non-volatile substance, *skatol-carbolyxic acid.* He isolated this in the following way. The organisms were grown on ordinary broth or peptone water (2—3%) for three or four weeks, and the cultures were then filtered through paper, and evaporated to ½—⅓ of their bulk. The fluid was then acidulated with acetic acid and saturated with ammonium sulphate. This process precipitated the proteoses, etc. which were filtered off, and the filtrate was then evaporated to dryness at 37° C. The dry mass was then ground to a fine powder and repeatedly extracted with boiling alcohol. The alcoholic extracts were mixed, the alcohol distilled off, and the residue dissolved in a small quantity of water, and extracted with ether. The skatol-carbolyxic acid being freely soluble in ether was found in the etherial solution. The tests for skatol-carbolyxic acid are given by Salkowski (*Zeitschr. f. physiol. Chem.* IX. 1885, pp. 8—23) as follows:

(1) Solutions of 1—1000 to 1—10,000 mixed with a few drops of pure nitric acid (sp. gr. 1·2) and then with a few drops of 2% potassium nitrite give a cherry red colour. This is easily soluble in acetic ether and amyl alcohol, but not in ether or chloroform. The solution of acetic ether on the addition of sodium hydrate becomes decolourised, and the soda solution becomes coloured; on acidulating with hydrochloric acid the acetic ether again takes up the colouring matter and the watery layer is decolourised.

(2) Solutions of 1—1000 mixed with an equal volume of hydrochloric acid and a few drops of 1—2 % chloride of lime give a purple red colour.

(3) Solutions of 1—1000 mixed with a few drops of strong hydrochloric acid and two or three drops of weak ferric chloride show a cherry red colour on heating.

Gas Production.

During its growth the diphtheria bacillus does not produce gas under any conditions.

Anaerobic Cultures.

It is generally stated that the diphtheria bacillus grows rather less freely in the absence of oxygen than in aerobic cultures. Hewlett and Knight (1897, p. 15) make the capacity for growth in hydrogen one of the distinguishing features between the diphtheria and the Hofmann's bacillus. They state that the former grows freely, but that the latter shows no growth. Peters, on the other hand, states that it does not grow anaerobically in an atmosphere of hydrogen.

Vitality in Culture.

Under favourable conditions the diphtheria bacillus remains alive in cultures for many months. Nedrigailoff (1901), for example, found that the diphtheria bacillus retained its virulence and vitality when kept on serum in sealed tubes for four years. Loeffler (1890) observed that cultures remained alive on gelatin for 330 days, and Abel (1893) succeeded in obtaining growths from three old dried agar cultures after 213, 212, and 169 days respectively. A fourth culture, however, tested after 163 days, was dead. For the purpose of preserving cultures sugar-free gelatin is a good medium. The tubes should be kept at room temperature, and protected from the light. Kept at 37° C. cultures die rapidly, usually within a few weeks.

Chromogenic Cultures.

It has already been pointed out that more or less coloration of the colonies is fairly common in cultures grown on serum, especially alkalised serum. Many examples occur which show this colour distinctly, especially after a few generations. Hill (1903) observed that six cultures which were kept growing on serum for 10 months showed a distinct increase in the yellow coloration. After a year's subcultivation one was remarkable in its cultural appearance, though typical in morphology and virulence. Cultures on serum showed a bright yellow colour in three days, but on agar after the same time they showed the ordinary whitish colour. The colour was soluble in chloroform, but the pigment

dried by evaporation was not soluble in water. Hill also observed feebly red coloured cultures which, when old, became a dirty, or even a dark, brown.

Abbott (1891) recorded a non-pathogenic diphtheria bacillus which gave rise to a coloured growth on potato. This organism, therefore, probably belonged to the group described in Chapter X.

The Influence of Temperature in Relation to the Growth of Diphtheria Bacilli on Artificial Media.

Diphtheria bacilli begin to develop at a temperature between 18—19° C., but growth is then very slow. They grow more rapidly as the temperature is raised, and attain their maximum development at about 35°—37° C. Roux and Yersin (1890) found that young cultures exposed to a temperature of 40° C. died in four days. According to Park (1900), however, development still occurs between 40—41° C., and he states that at this temperature the organisms may retain their virulence for months.

A. Powers of Resistance of Diphtheria Bacilli from Cultures.

(1) *Thermal death point.* According to the earlier observers, Klein (1889), Welch and Abbott (1891), Zarniko (1890), and Roux and Yersin (1890) the thermal death point with 10 minutes' exposure in capillary tubes is 58° C., and this has been confirmed by all the subsequent observers.

(2) *Effects of Cold.* Abel (4. v. 1895) found that cultures protected from the light and left in the open during the winter, when the temperature on some occasions fell as low as −23° C., kept alive as long as the experiment was carried on; in some cases up to 86 days. Kasansky (1899) was able to confirm these observations, and in one case found that a culture exposed at times to −25° C. was alive at the end of 118 days. Testi (1902) froze to −20° C. and heated to 37° C. for 30 minutes alternately (twelve times) emulsions of diphtheria bacilli in water, and found that cultures subsequently made from them were alive and virulent. Ravenel (10. vi. 99) immersed silk threads, impregnated with a suspension of diphtheria bacilli in water, in liquid air (−190° C.) without drying. Cultures made from the threads after 15 and 30 minutes' exposure gave vigorous growths.

Macfadyen (1. ii. 00) later cooled cultures of diphtheria bacilli to the temperature of liquid air (−190° C.) for 20 hours without effect. Macfadyen and Rowland (5. iv. 00, pp. 339, 488) made further experi-

ments. They exposed emulsions hermetically sealed in fine capillaries to a temperature of −190° C. for seven days, and to −252° C. for 10 hours without any appreciable effect.

(3) *Effects of Drying.* Hill (1902) dried diphtheria bacilli from agar cultures on glass rods, and left them in a box at laboratory temperature in diffuse light, and with slow diffusion of air. Cultures made from these rods showed that the times at which the organisms died varied greatly, some being dead after seven days, and others alive after 20 days.

Put into tabular form his results were as follows:—

No. of rods exposed	20	22	20	22	22	19	99	22
No. of days of drying	1	2	7	7	10	14	14	20
Positive results	20	22	16	13	12	6	9	2
Negative results	—	—	—	3	5	10	77	15
Contaminated	—	—	4	6	5	3	13	5

Abel (1895) dipped silk threads in the condensation water of cultures (three strains) and dried them over acid. Some of these were kept in the open during winter and some in the room. From time to time cultures were made on serum from these threads. Under both conditions the organisms on these threads were found to be alive up to 56 and even 86 days. Their virulence was not lessened.

Deycke (1898) made observations on the length of life of diphtheria bacilli obtained from agar and broth cultures dried on lime wash, oil paint, and glue covering wood and cement.

The following table is a summary of his results:—

Material	Materials smeared with bacilli from agar culture. Final living growth of bacilli obtained in	Materials smeared with broth culture. Final living growth of bacilli obtained in
Oil paint on wood	4 days	24 hours
Lime ,, ,,	9 ,,	3 days
Glue ,, ,,	18 ,,	3 ,,
Oil paint on cement	24 hours	2 days
Lime ,, ,,	10 days	4 ,,
Glue ,, ,,	10 ,,	5 ,,

Reyes (1895) compared the resistance of diphtheria bacilli placed on various materials in a moist and dry condition kept in the dark and in the light.

Diphtheria bacilli placed on	Kept in the dark		Kept in the light	
	In dry condition	In moist condition	In dry condition	In moist condition
Silk are dead in	5 days	8 days	4 days	6 days
Paper ,, ,,	4 ,,	8 ,,	3 ,,	5 ,,
Linen ,, ,,	12 ,,	18 ,,	5 ,,	15 ,,
Mud ,, ,,	100 ,,	120 ,,	74 ,,	120 ,,
Sand ,, ,,	18 ,,	34 ,,	5 ,,	34 ,,

He finds that by rapid drying over sulphuric acid they are killed at the latest in 48 hours.

Golowkow (1895) made experiments of a similar nature. He took pieces of linen, cloth, and satin, and sterilized them at 120—130° C. These were infected by means of broth cultures, and some were left in the dark and others in the light at room temperature. After certain times the pieces were put into broth and cultivated. He found the bacilli dried on cloth were dead after 13 days, and on linen after 16 to 23 days in the dark, and after 20 days in the light. On a certain gray cloth they were alive and capable of growth after 26 days. On unwashed gray linen the bacilli were dead after 24 hours, but on the same material washed they were living after 20 days. The author thinks this is probably due to some chemical substance in the colour or finish. He states that the virulence of the diphtheria bacillus is diminished by drying.

Germano (1897) also came to the conclusion that diphtheria bacilli remain alive a long time on cloth.

The observations which have just been quoted prove that diphtheria bacilli from cultures, without the protection which portions of membrane and saliva can give them, are capable of remaining alive after drying for very considerable periods.

(4) *Effects of Light.* It is well known that diffuse daylight hinders the growth of diphtheria bacilli even when other conditions are favourable, and that it shortens their period of life in cultures. Direct sunlight has a much more powerful bactericidal influence. Gehrke (1899), for example, found that suspensions of the bacilli in clear water were killed by direct sunlight in 2—8 hours, whereas in water yellow with organic material they remained alive longer. He found that agar cultures were killed in six hours, but that broth cultures, one or two days old, were very resistant to its influence. Ledoux (1895), however, states that even broth cultures are killed within a few days. He also found that bacilli dried in thin layers were rapidly killed by direct sunlight.

(5) *Period of life in soil and dust.* Reyes (1895) found that diphtheria bacilli enclosed in dry mud and kept in the dark remained alive for 100 days, in moist mud in the dark for 120 days, in dry mud exposed to light for 74 days, and in moist mud kept in the dark for 120 days. In dry sand kept in the dark they remain alive for 18 days, but in dry sand exposed to the light for only five days. In moist sand, whether kept in the dark or exposed to the light, they

remain alive up to 34 days. Leighton (1901) found that diphtheria bacilli were still capable of producing a growth after being enclosed for 18 days in clay. The clay was kept moist and warm, and studied periodically. Germano (1897) came to the conclusion that diphtheria bacilli can withstand drying in dust for a long period.

(6) *Putrefaction.* Klein (31. v. 1899) made some observations on the bodies of guinea-pigs dead of experimental diphtheria, which tend to show that under certain conditions diphtheria bacilli can resist the changes due to early putrefaction. Some of these bodies were buried in the earth direct, others in sand, and some in wooden coffins. After 14, 21, and 31 days' burial animals buried in each of these ways were exhumed, and cultures made on Kanthack's ascitic fluid medium from the juice of the swollen inguinal glands. All organisms resembling diphtheria bacilli were proved by cultural and animal inoculation tests. After 14 days the bacilli were alive in all cases, but no diphtheria bacilli could be found after 21 and 31 days' burial.

(7) *The effects of Antiseptics.* Owing to the lack of uniformity in the methods used and the time limit adopted by various observers it is difficult to compare the actions of various antiseptics on the diphtheria bacillus. In the following table is given the action of various substances on broth cultures or fresh emulsions of diphtheria bacilli, under either of which conditions the organisms are easily affected by the reagents.

Table showing the action of some common germicides on the diphtheria bacillus in fluid cultures.

Acids—Carbolic 1 : 100	Kills within 10 minutes	(Smith and Somerville, 1905).
Hydrochloric 1 : 25	,, 10 ,,	
Nitric 1 : 50	,, 10 ,,	
Sulphuric 1 : 10	,, 10 ,,	
Alcohol (98—30 %)	,, 1 minute	(Russ, 1905).
Ammonia (gas)	,, 4 hours	(von Rigler, 1893).
Chlorine 1 : 10,000	,, 10 minutes	(Williams, 1895).
Creolin 1 : 2000	,, 10 ,,	(Smith and Somerville, 1905).
Cyllin 1 : 800	,, 4 ,,	(German, 1905).
Formalin 1 : 100	,, 15 ,,	
Lysoform 1 : 100	,, 25 ,,	
Mercuric chloride 1 : 5000	,, 10 ,,	
Tricresol 1 : 33	,, 10 ,,	

From such experiments nothing, however, can be deduced as to the action of these germicides on the bacilli in the dried state, or when

enclosed in masses of membrane or mucus. Russ (1905), for example, has shown that 98% alcohol does not affect the vitality of diphtheria bacilli dried on silk threads after an exposure of an hour, but kills the bacilli in emulsions within a minute. 30—60% alcohol on the other hand kills the dried bacilli in five minutes, and the bacilli in emulsions in one minute. Mercuric chloride (1—1000), potassium permanganate (1—20), and carbolic acid (1—20) kill thick layers of cultures in 20 seconds (Bowhill, 1899).

B. Powers of Resistance of Diphtheria Bacilli enclosed in Membrane.

It has long been known that in dried pieces of membrane diphtheria bacilli remain alive for long periods of time. Loeffler (1890) observed living bacilli in fragments of dried membrane after 14 weeks, Park and Beebe (1895) after 17 weeks, and Roux and Yersin (1890) found them still alive in pieces of membrane kept in the dark at room temperature after five months. Many other later observers have confirmed these statements. Even when enclosed within pieces of membrane, the diphtheria bacilli are considerably influenced by the external conditions. The presence of light, moisture, or a high temperature may cause them to die more rapidly than when the conditions are more favourable. Roux and Yersin (1890), for example, found that no living bacilli could be obtained from a dry membrane hung up in the open and exposed to the air, sun, and rain after one month. According to these observers the bacilli dried in membrane can resist a temperature of 98° C. for one hour.

The Behaviour of Diphtheria Bacilli in certain Food Substances.

(*A*) *Water.* The period of the vitality of the diphtheria bacillus in water depends on whether the water is pure or contains nutritive substances, on the treatment it has received, and on the external conditions. According to Montefusco (1896) the diphtheria bacillus remains alive in ordinary water for 20 days, and in sterilized water for 45 days. Seiler and Stoutz (1904) thought that it multiplied in sterilized water up to 9—12 days. In distilled water however, according to the observations of D'Espine and de Marignac (1890), it dies within 24 hours. In strongly polluted water the diphtheria bacillus only remains alive six days (Montefusco). Gehrke (1899) found that the action of sunlight on diphtheria bacilli suspended in water differed

according to the degree of pollution of the water. Whereas the bacilli suspended in pure water died in 2—8 hours, they died much more slowly in water yellowish with organic material.

(*B*) *Milk.* The most extensive experiments on the growth of diphtheria bacilli in various kinds of milk have been made by Schottelius (1896). He compared the growth and multiplication of diphtheria bacilli in broth and in raw and sterilized milk, both at room temperature and at 37° C. He found that the multiplication of the bacilli was greatest when tested after 6, 24, and 48 hours' growth in raw milk, and greater in broth than in sterilized milk both at 37° C. and at room temperature. In one of his experiments he added 1 c.c. of a broth culture to 20 c.c. of sterilized milk, 20 c.c. of milk taken straight from the udder of the cow, and to 20 c.c. of broth.

The actual numbers found per c.c. after growth for six hours were :—

	At room temp.	At 37° C.
In raw milk	21,280,000	50,160,000
In sterilized milk	2,280,000	6,080,000
In broth	7,600,000	18,240,000

Eyre (1904) also showed that the rate of multiplication of diphtheria bacilli in milk, obtained as sterile as possible from the udder of the cow, is very great.

	0 hours	24 hours	48 hours	7 days
Diphtheria bacilli	39	1,170	22,000	19,000,000 per c.c.

The conclusions of Montefusco (1896) and Rubinstein (1904) are, however, not in agreement with these experiments. Montefusco came to the conclusion that sterilized milk was a good medium for diphtheria bacilli, but that no further growth took place in raw milk after three days, while Rubinstein says that in raw milk they die in 24 hours. He states, however, that in prepared butter-milk, as given to children, they remain alive for five to seven days.

Klein (1900), on the other hand, stated that in sterilized milk diphtheria bacilli showed no growth at 37° C. in 14 days, but at 20° C. showed copious growth. He also failed to detect growth in sterilized *cream* at either 37° C. or 20° C.

(*C*) *Butter.* According to Montefusco (1896) diphtheria bacilli remain alive for two days in butter, but even after six hours have begun to lose their virulence.

(*D*) Klein (1900) found that diphtheria bacilli did not grow on a sterilized block of *cheese* either at 37° C. or 20° C.

(*E*) *Wines* according to their acidity are more or less noxious to diphtheria bacilli (Montefusco, 1896).

(*F*) On *fresh bread* the bacilli are alive after 24 hours, but are dead after 48 hours. On stale bread they live longer (Montefusco, 1896).

Virulence.

The typical virulent diphtheria bacillus when injected into the subcutaneous tissues of a guinea-pig causes the death of the animal within three or four days with certain characteristic lesions. At the autopsy there is found in the subcutaneous tissue at the site of inoculation a whitish necrotic area which varies in size from about ·5 to 2 cms. in diameter. A more or less extensive, often haemorrhagic, gelatinous oedema surrounds this area. Sometimes the oedematous area is small, at other times it occupies most of the surface of the abdomen, and not infrequently it extends over the whole ventral surface, including the axillae, groins, and neck.

On opening the peritoneal cavity the vessels of the omentum and mesentery are generally found to be somewhat congested. Some congestion of the liver, kidneys, and spleen is also common. The most striking and constant feature is, however, the intense congestion of the vessels of the suprarenal capsules. The colour of these organs is changed from the yellow of their normal condition to pink, red, or a very dark deep red. A small quantity of fluid is sometimes found in the peritoneum.

On opening the thorax a quantity of clear straw-coloured fluid is frequently found in the pleural cavities. The quantity of this fluid may vary between traces which cannot be measured up to 15 c.c. or more. The lungs may be normal or congested.

The histology of these lesions and the rarer conditions are dealt with later (Chapter VII). In 1897 Theobald Smith wrote, "We may, for convenience, regard the disease producing power of diphtheria bacilli as made up of two elements, toxicity and virulence. The former represents the rate of accumulation of toxin in culture fluids, and is easily measured; the virulence, on the other hand, which may be regarded as the behaviour of diphtheria bacilli towards living tissue, is as yet an unknown quantity." The toxin producing power is fully dealt with in another section (Section V), and it is only the virulence with which the present section is concerned. A culture of the diphtheria bacillus is said to be fully virulent when it is capable of

killing a half-grown guinea-pig after subcutaneous inoculation within three, or at the outside four, days, with the lesions just described. It has hitherto been generally considered that diphtheria bacilli of all degrees of virulence are to be met with from the fully virulent to the totally non-virulent, which even in large doses produce no effect on guinea-pigs. In fact Welch (1891) summarised his conclusions on this point as follows: "It is to be said that this bacillus, as obtained in pure culture from different cases of diphtheria, varies in virulence, as tested upon animals, to a greater degree than any known pathogenic organism," and Abbott (1902, p. 400) more recently concluded his statements on this subject as follows:—"Under certain circumstances with which we are not acquainted *Bacillus diphtheriae* becomes diminished in virulence, or may lose it entirely, so that it is no longer capable of producing death of susceptible animals, and may cause only a transient local reaction, from which the animal entirely recovers. Sometimes this reaction is so slight as to be overlooked, and again careful search may fail to reveal evidence of any reaction at all. This exhibition of the extremes of its pathogenic properties, viz., death of the animal on the one hand, and only very slight local effects on the other, was at one time thought to indicate the existence of two separate and distinct organisms that were alike in cultural and morphological peculiarities, but which differed in their disease producing power. Further studies on this point have, however, shown that the genuine *Bacillus diphtheriae* may possess almost all grades of virulence, and that absence of, or diminution in, virulence, can hardly serve to distinguish as separate species those varieties which are otherwise alike; moreover, the histological conditions found at the site of inoculation in animals that have not succumbed, but in which only the local reaction has appeared, are in most cases characterised by the same changes that are seen at autopsy in animals in which inoculation has proved fatal." Most writers seem to agree with these opinions, and many observers have proved that the degree of virulence has no relation to the severity or mildness of the disease in the patient from whom the culture was obtained. There can then be but little doubt that variations in virulence are to be found amongst diphtheria bacilli, nevertheless there are many reasons for thinking that intermediate degrees of virulence between full virulence and total lack of virulence are much less common than has been generally supposed. In many of the experiments which are quoted to illustrate these intermediate degrees of virulence the conditions are not uniform.

Some investigators had cultivated the bacilli preparatory to inoculation on ordinary broth containing various quantities of muscle sugar, and some even on broth to which glucose had been added; others have used bacilli from solid cultures emulsified in salt solution, or the water of condensation. The times during which the cultures have been allowed to grow, the dose, and the size of the animals have also all been subject to variation. In fact Theobald Smith (1897) went so far as to say that "the large amount of labour which has been expended in comparing the virulence of diphtheria bacilli from different sources is all but wasted."

Some of these sources of possible error are discussed below. The effect of the presence of sugar in the medium on the toxin production and vitality of the bacilli has already been mentioned (p. 162). Cultures which have become acid frequently produce no result on guinea-pigs, although the strain from which they have been sown is highly virulent. It is therefore of importance that the broth used for cultivating the bacilli previous to injection should be free from sugar.

In most experiments the organisms are grown in broth for 48 hours and certain quantities of the culture are then injected. Most experimenters have not, however, paid any attention to the relative degree of growth of the various strains, although it is well known that many strains, which have been recently separated, grow poorly in broth at first, but after one or two transplantations become accustomed to the medium and grow well.

Cobbett (1901) found that three strains in doses of ·1 c.c. of 48 hour cultures failed to kill, but these cultures were poorly grown, and when injection was repeated a few days later, after the culture had passed through one or more generations in broth and had become accustomed to this medium, death took place within 48 hours. The writer (1904) had a similar experience, but found one culture of diphtheria bacilli which even after several transplantations grew poorly in broth, and only killed animals after 12 days. Williams (1902) also found certain bacilli which immediately after isolation showed very scanty growth in broth, and says that "such broth cultures gave very little reaction in guinea-pigs except in large amounts, though the bacilli themselves when inoculated from serum cultures were decidedly virulent. In ascitic broth however, where they grew rapidly and abundantly, they showed a high degree of toxicity." After ascitic broth was used to test the virulence, all the specifically virulent diphtheria bacilli, about 100 cultures, segmented and granular, were

found to be highly pathogenic for guinea-pigs, the largest dose required to produce death being ·2 c.c. and the average ·01 c.c.

Half-grown guinea-pigs (150—350 grms.) are generally employed for the virulence test, the larger animals being somewhat more resistant. It has generally been assumed that all guinea-pigs are about equally susceptible, but Theobald Smith (1897), in estimating the absolute toxicity of culture filtrates, came to the conclusion that animals from some sources are much more susceptible to diphtheria toxin than from others. For several years this writer had been experimenting only upon guinea-pigs reared under his supervision. During this time all animals used exhibited a remarkably uniform susceptibility. Latterly (1905) the guinea-pigs purchased from a dealer had to be used, and it was soon evident that for them the minimal fatal dose was about $\frac{1}{2}$—$\frac{3}{5}$ of that to which the home-bred guinea-pigs succumbed. In seeking some cause for these irregularities in susceptibility he found that the differences were due to family inheritance, and showed that all individuals from a litter possess the same degree of susceptibility or resistance, and that several litters of the same mother are the same in this respect. Further, he found that the darker animals (black, and black and red) were able to stand 10% more toxin than the white ones. Behring (1898) states that Ehrlich obtained from a dealer a race of guinea-pigs showing relatively great resistance, and Anderson (1906) has recently shown that female guinea-pigs, which have been treated with toxin, in many cases (50%) transmit a considerable degree of immunity to their young.

In experiments relating to virulence the statement is frequently made that the animals have died some considerable period after the injection without any symptoms of paralysis, and death is attributed to the inoculation. Theobald Smith (1897) observed that a certain number of guinea-pigs became emaciated some time after full recovery. They grew literally smaller and moved about the cage with arched backs, and the bones could be felt through the skin. In all such cases after they had been chloroformed the kidneys were found remarkably pale, and in some instances perhaps a little enlarged. He does not believe that this condition is due to the toxin, but that it is peculiar to, or at any rate not uncommon in, the guinea-pig living in confinement, as the disease was found in both young and old untreated guinea-pigs.

The experimental animals must also be kept under good conditions and be free from disease. Valagussa and Ranelletti (1898) attempted

to render the conditions of their animal experiments as comparable as possible to the conditions in which the poor live. They kept animals under various conditions of fatigue and hunger, and on a poor and short diet, and in foul air. They found that under the influence of these factors the toxic strength of the diphtheria products was heightened and that the animals succumbed more quickly and with more marked lesions. Previous inoculation with the products of saprophytic and pathogenic organisms also weaken the powers of resistance.

It follows from what has just been stated that in attempting to determine the virulence of various strains of diphtheria bacilli the conditions of the experiments ought to be as equal as possible. Cultures of the bacillus, which have become accustomed to the medium and grow well, incubated at 37° C. in sugar free broth for 48 hours, should be used and injected subcutaneously in small doses. Although broth kept under the influence of light and air undergoes changes, in a series of experiments the results are more likely to be comparable if the same broth is used throughout. The variation in the susceptibility of the animals is probably not a factor of much account in testing the virulence, but should be borne in mind. Half-grown guinea-pigs seem the most suited for these experiments.

Under the conditions just quoted very few series of extended observations have been made.

Cobbett (IV. and X. 1901) in two outbreaks at Cambridge isolated 79 cultures of diphtheria bacilli. Of these 11 were totally non-virulent, and the rest were fully virulent killing in doses of ·1 c.c. within two or three days. He found none with intermediate degrees of virulence.

Williams (1902, p. 103), as already mentioned (see p. 175), found no intermediate degrees of virulence. She says: "After ascitic broth was used to test toxicity, all the specifically virulent diphtheria bacilli,—about one hundred cultures,—segmented and non-segmented varieties, were found to be highly toxic for guinea-pigs. The largest dose of a two to six day culture on ascitic broth which was required to produce death in this animal was one-fiftieth cubic centimeter, the average dose being one-hundredth cubic centimeter."

Graham-Smith (1904) during an epidemic isolated the diphtheria bacilli from 113 out of 117 persons, patients and contacts, who were found to be harbouring them. Of these 87 were fully virulent killing in doses of ·2 c.c., and 25 non-virulent, producing no effect in doses of 2 c.c. One only killed after 12 days, but this strain, even after repeated transferences, grew very poorly in broth. As this is the largest series

from an epidemic in one place yet examined in this way the results of the injections and autopsies are given in full (Chapter XII).

Theobald Smith and Walker (1896) used the relative accumulation of toxin in culture, eliminating the bacilli by filtration, in comparing the disease producing power of diphtheria bacilli. In giving their reasons for making use of this method Smith says: "The writer is fully aware of the fact that but an instrument of pathogenic power is here dealt with, and under artificial conditions, since we do not know the nature of the nutritive fluid which the bacilli make use of on mucous membranes, nor as a consequence, whether the toxin production in bouillon is a true index of the production of toxin on mucous membranes. The problem is in fact very complex, as with all infectious diseases, and all we can hope to do at a time is to examine one factor of disease as carefully as possible, while eliminating all the others for the time being. The use of living cultures upon animals is of no service in these experiments because it produces at once three variable factors: (1) the bacilli as potential toxin-producers after injection; (2) the poison of their bodies after destruction; and (3) the toxin preformed in the culture fluid injected."

These observers estimated the toxicity of the culture fluid from 42 races of diphtheria bacilli obtained from different localities under equal conditions. The minimal fatal dose varied from ·036—·12 c.c., that is to say, there was considerable uniformity in the toxin producing power, and they came to the conclusion that this power is not so variable as it has generally been considered. They also investigated in the same way cultures isolated from patients at various times after the disappearance of the membrane. There was amongst these also a considerable uniformity in the production of toxin. Cultures of much greater toxin producing power have been isolated, but some of these at any rate have increased in their capacity for producing toxin by prolonged cultivation.

Park (1900) on the other hand states that "the virulence of diphtheria bacilli from different sources, as measured by their toxin production, varies enormously. Between bacilli which produce a great deal of toxin, and bacilli which apparently produce none, we find all grades of virulence."

Summary of virulence experiments.

The pathogenic properties of the diphtheria bacillus can be tested in two ways (1) by the injection of the sterile products of the organism, or (2) by the injection of living cultures. The former method involves a considerable amount of labour, and has the objection that but an instrument of pathogenic power is dealt with. The latter is the method which has been most frequently used, but many objections have been urged against it: that the conditions are artificial, in that subcutaneous inoculation bears little resemblance to the ordinary mode of infection in man, and that the conditions have varied in almost every series of experiments, and in the individual experiments of each series. Nevertheless, if care be taken to equalise the conditions as far as possible, a correct estimate may be formed of the virulence of the bacilli towards guinea-pigs, and satisfactory evidence is yet lacking to show that races non-virulent to guinea-pigs can produce serious disease in man.

The great majority of workers consider that diphtheria bacilli obtained direct from the throats and noses of *clinical cases*[1] vary in virulence, as ascertained by the subcutaneous inoculation of living cultures, to an extraordinary degree: from fully virulent races which in small doses kill guinea-pigs within three days to races which produce no effect on these animals when injected in large doses. The conditions of most of these experiments have not, however, been uniform.

The results of those experiments which have been made under uniform conditions, i.e. the inoculation of well-grown 48-hour cultures in sugar-free broth or ascitic broth into half-grown guinea-pigs, show that the very great majority of races of diphtheria bacilli are either fully virulent or totally non-virulent for these animals. On further investigation it has usually been found that the latter races have been derived not from clinical cases but from infected contacts. In spite of the latter investigations there can be but little doubt that partially attenuated races do occur, but with much less frequency than is generally supposed.

Classification of diphtheria-like bacilli according to virulence.

Park and Beebe (1895) classified the diphtheroid bacilli commonly met with in the throat and nose according to their virulence and power of producing acid in broth:

[1] Experiments on the virulence of diphtheria bacilli derived from convalescent patients and infected contacts are given later (pp. 231, 233).

[1]I. Bacilli identical in appearance both in culture and under the microscope with the diphtheria bacillus.

(*a*) Pathogenic acid producers = Virulent diphtheria bacilli.

(*b*) Non-pathogenic acid producers = Non-virulent diphtheria bacilli.

II. Bacilli somewhat resembling, but shorter and stouter than, diphtheria bacilli.

Non-pathogenic, non-acid producers = Hofmann's or the pseudo-diphtheria bacillus.

Although this classification does not include the more uncommon forms of diphtheroid bacilli, which have from time to time been encountered, it clearly distinguishes between the forms which are of common occurrence in diphtheria investigations. Had this scheme been more frequently adopted much of the confusion might have been avoided, which has arisen owing to the use of the term pseudo-diphtheria bacillus to denote both the non-virulent diphtheria bacillus and the Hofmann's bacillus.

The Distribution of the Diphtheria bacillus.

I. The occurrence of diphtheria bacilli in notified persons.

Novy (1895) gives a table showing the results of observations by European workers between 1886 and 1895, in which 2846 cases of diphtheria were examined and diphtheria bacilli found 2344 times (82·4 %). European and American observers combined examined 8186 cases finding the specific bacillus in 5943 (72·6 %). French investigators in the Pasteur Institute obtained the bacillus in 701 out of 960 cases (73 %), and during 1894 certain German workers satisfied themselves of the presence of the bacillus in 945 out of 972 cases, giving a percentage of 97·2. The work of Park and Morse showed that out of 5340 suspected cases 67·5 % were true diphtheria.

Woodhead (1896) states that of the 12,172 cases admitted into the Metropolitan Asylums Board Hospitals during 1895–6, and certified as suffering from diphtheria, at least 20 %, or about 3000, offered no bacteriological evidence of diphtherial infection.

Josias and Tollemer (1903) found that of 709 cases admitted into hospital diagnosed as clinical diphtheria, only 580 (81 %) showed bacteriological evidence of the disease.

[1] Cobbett's (IV. 1901, p. 244) tabulation of Park and Beebe's classification is shown above.

The following table summarises the observations of the Massachusetts State Board of Health for the nine years ending March 31st, 1905:

Clinical diagnosis	Bacteriological diagnosis			Percentage of true diphtheria
	Positive	Negative	Doubtful	
4113 positive	2555	1504	54	61·6
2340 negative	421	1880	39	18·0
2971 doubtful	982	1942	47	33·0
2031 not given	734	1247	50	36·1

The results of the bacteriological examinations of the 30,000 certified cases quoted above show that bacteriological evidence of diphtheria was present in only 71 %. The experiences of most other observers who have examined smaller numbers of cases are in accord with this.

Some of the diseases clinically simulating diphtheria and occasionally mistaken for the latter are given later (Chapter XI).

II. The distribution of diphtheria bacilli in healthy persons, who have been recently exposed to the disease.

Many of the statistics, which have been compiled and quoted, on the distribution of the diphtheria bacillus in healthy persons have been based on the morphological appearances of the bacilli obtained from cultures alone, without any attempt to isolate and test the suspicious organisms. Very few observers have tested the virulence of even a small proportion of the diphtheria bacilli they have observed in such cultures, but many have assumed that all the morphologically typical bacilli were virulent.

Although it is of the utmost importance to ascertain whether virulent diphtheria bacilli ever occur amongst normal persons, who have not been exposed to diphtheria, and although all observers are agreed that virulent and dangerous diphtheria bacilli occur in the mouths of certain healthy persons, who have contact with the sick, yet not infrequently it is difficult to ascertain whether the persons examined by some investigators have been recently in contact with diphtheria or not.

In the following summary of observations an attempt has been made to classify the various observations, which have been made on healthy persons, most importance being attached to the work of those experimenters, who have tested the organisms they have found, and made careful inquiries as to the possibility of recent contact.

The proportion of "contacts," or persons who have recently been in intimate connection with the disease, who become infected with the diphtheria bacillus to those who do not become infected, is subject to great variation according to the investigations of different observers. To some extent these differences probably depend on the measures taken to promptly isolate the sick, the class of persons examined, and the views of the observer as to the importance of the bacilli which he meets.

Contacts have here been divided into classes according to the closeness of their relationship to the diseased persons:—

(*a*) Family contacts, or members of the family to which the diseased person belongs. (*b*) Persons attending on the sick. (*c*) Persons in close connection with the sick in hospital wards or institutions. (*d*) Scholars of infected schools. (*e*) Contacts not included in the above classification.

(*a*) *Infected families.*

Cobbett (IV. 1901) discovered diphtheria bacilli in all the healthy members of one family. The bacilli from three of them were isolated and found to be virulent (100%). Park (1892) quotes the case of a family of four persons, all of whom suffered from diphtheria and of whom three died. The bacilli in all cases were virulent (100%). Scheller (1905) recently examined 284 relatives of diphtheria patients and found diphtheria bacilli in 108 (38%). Three infected families which he carefully examined contained 7, 6 and 3 healthy individuals, and of these 16 persons 14 were found to be harbouring diphtheria bacilli within a few days of the outbreak of the disease (87·5%). In these families the distribution of the bacilli was extremely interesting, and one example is given below in detail:

		Date of examination in days from the outbreak of the disease						
Age	Clinical diagnosis	2	22	31	37	46	51	58
7 years	Diphtheria	+	+	+	0	+	0	0
1½ ,,	Healthy	0	0	0	0	0	0	0
2¾ ,,	,,	0	0	0	+	0	0	0
8 ,,	,,	+	+	+	+	0	+	0
9 ,,	,,	+	0	+	+	+	0	0
17 ,,	,,	0	0	0	0	0	+	0
31 ,,	,,	+	0	+	+	0	0	0
39 ,,	,,		+	+	0	0	0	0

+ = diphtheria bacilli present in cultures. 0 = no diphtheria bacilli found in cultures.

Spirig (1899) examined the children of two families numbering four and six respectively. There was one case of diphtheria, and of the remaining nine children six were found to be harbouring diphtheria bacilli (66·6 %). Of these six, five subsequently developed the disease. Williams (1896), in an infected family of five persons, found three with diphtheria bacilli (60 %). Park and Beebe (1894) amongst 48 children in 14 infected families found diphtheria bacilli in 50 %. All the cultures tested were virulent. In other families, however, in which isolation was as perfect as possible only 10 % were found to be infected. Kober (1899) made bacteriological examinations of the throats of 128 persons, relatives of patients, and found diphtheria bacilli in 15 (11·7 %).

In this class of contacts, therefore, the proportion of infected persons varies between 100—50 % in families in which strict isolation is not observed, but may be as low as 10 % under the best conditions.

(*b*) *Persons attending on the sick.*

Numerous examples are to be found of physicians, nurses, and students becoming infected with diphtheria bacilli from patients, but very few observations show what proportion of these persons becomes infected, and in any case the degree of infection under these conditions must depend greatly on the precautions used.

Richmond and Salter (1898) examined the throats of 129 doctors, nurses, and medical students who had diphtheria patients under their charge, and found diphtheria bacilli in 62 of them (48 %).

Pugh (1902) examined the throats of 56 nurses and found diphtheria bacilli in 7 (12·5 %).

(*c*) *Persons in close connection with the sick in hospital wards and institutions.*

Lister (1898) in the Shadwell hospital examined 125 children, 69 of whom had nasal discharges. Organisms resembling diphtheria bacilli were found in the noses of 61 of them (48 %). 69 out of 242 cultures taken from the throats of the inmates of an institution known as the Bethany Home (Minnesota Board of Health, 1900) showed diphtheria bacilli at a time when there were three clinical cases (28 %). Müller (1897) systematically examined the children (100) in a general ward in Berlin. Four had diphtheria bacilli when the examination began and six were later admitted with them. The latter were all recent contacts. 14 children acquired the bacilli during their stay (16 %).

He found that infection passed from cot to cot. Soerensen (1898–9) during three years examined the patients in scarlet fever wards. During this period 2290 patients passed through the wards amongst whom 54 harbouring diphtheria bacilli were admitted, and these gave rise to 49 cases of clinical diphtheria and 274 (12·5 %) infected contacts. Graham-Smith (1904) out of 48 patients and nurses examined in a general ward found 5 contacts with virulent diphtheria bacilli and one with non-virulent (12 %). Park and Beebe (1895) found 6 out of 55 children in the New York Foundling Hospital to be harbouring diphtheria bacilli. Of these organisms 5 were virulent. Some cases of diphtheria had from time to time occurred in this institution. (Virulent bacilli 9 %.) Gross (1897) examined weekly the throats and noses of all the children in the Boston City Hospital during a period of six months. 316 children were examined, of whom two suffered from diphtheria. Of the 314 children who showed no clinical manifestations of diphtheria 24 at some period gave cultures of diphtheria bacilli (7·6 %). In the State School for Feeble-minded, Faribault (Minnesota Board of Health, 1900), 3 out of 50 persons examined showed diphtheria bacilli (6 %). In this institution there had been no clinical diphtheria for four months. Chatin and Lesieur (1900) made observations on 75 children in an asylum in which there had been one case of diphtheria. 14 of the children were suffering from sore throats, of whom two had diphtheria bacilli (2·66 %); the remaining 61 were free. Johannessen (1895) found virulent diphtheria bacilli in the throats of 7 out of 38 healthy contacts in a hospital ward.

(*d*) *Scholars of infected schools.*

Crowley and Eurich (1904) examined 93 cultures taken from the throats of the teachers and children of the infant department of a school in which 80 cases of diphtheria had occurred. 42 were found to be infected with diphtheria bacilli (45·1 %). Peck (1901), during an outbreak in a boarding-school, found 31 infected contacts amongst the 100 scholars (31 %). From a school in which several cases had occurred 63 children were examined by Cobbett (x. 1901) and 13 infected contacts found (20 %). Cultures from 10 of these were isolated and tested and 6 turned out to be virulent and 4 non-virulent. In a previous outbreak (iv. 1901) he had examined 650 persons, mostly school children, and discovered diphtheria bacilli in 19. These latter were either children attending the school most affected, or were inmates of houses where

there were actual cases. The examination conducted for the State Board of Health of Minnesota (1901) in certain schools resulted in the following findings:—

School	Children examined	Typical diphtheria bacilli in	
1. Albert Lee	24	6 (25 %)	Diphtheria prevalent.
2. Elbow Lake	74	15 (20 %)	Diphtheria prevalent.
3. Faribault	57	6 (10 %)	Diphtheria had been prevalent.
4. Owatonna public schools	40	2 (5 %)	One case of diphtheria known in the town.
5. Mankato	30	1 (3 %)	Cases recently in the school.

Berry and Washbourn (1900) examined bacteriologically 148 girls and teachers with abnormal throats living in a school in which several cases of diphtheria had occurred, and discovered diphtheria bacilli in the throats of 19 (12·1 %). Denny (1900) examined 190 boys in a truant school where there were 10 cases of diphtheria, and found 16 with diphtheria bacilli. Of these 15 lived in one house (9 %). Gabritschewsky (1901) in one school outbreak examined 66 healthy children, and found that 21 of them were harbouring diphtheria bacilli (31·8 %). After another school outbreak he examined 230 scholars on their return to the school, which had been closed for two months. 10 harbouring virulent diphtheria bacilli were discovered (4·3 %). Graham-Smith (1902) in an epidemic at Colchester found 54 (10·4 %) persons out of 519 examined harbouring diphtheria bacilli. All these persons were school children or persons connected with schools. Morphological and cultural methods were relied on, as no tests for pathogenicity could be undertaken there. In 1904 Graham-Smith examined several schools during an outbreak of diphtheria at Cambridge with the following results:—

School	No. of persons examined	Notified persons with diphtheria bacilli				Contacts infected with diphtheria bacilli			
		Virulent		Non-virulent		Virulent		Non-virulent	
St Matthew's School									
Infants (March)	317	13 (4·1 %)	4·3 %	0		12 (3·7 %)	4·1 %	4 (1·2 %)	·9 %
,, (Nov.)	59	6 (10·2 %)		0		4 (6·7 %)		0	
Girls	132	3 (2·2 %)		0		5 (3·8 %)		1 (·7 %)	
Catherine Street School									
Class i	47	0	·6 %	0		0	3·1 %	0	·9 %
ii	103	0		0		1 (·9 %)		0	
iii	64	2 (3·1 %)		0		7 (10·9 %)		1 (1·5 %)	
iv	63	0		0		2 (3·1 %)		2 (3·1 %)	
v	41	0		0		0		0	
Sturton Street School	125	3	2·4 %	0		2	1·6 %	1	
Abbey School	33	0		0		0		1 (3 %)	
New Street School	43	0		0		0		1 (2·3 %)	
Park ,, ,,	20	1 (5 %)		0		0		0	
Ross ,, ,,	47	1 (2·1 %)		0		0		0	

Thomas (1904) during 29 school outbreaks examined 1027 children from infected classes in the public schools, and discovered amongst them 77 with diphtheria bacilli (7·5 %). Fibiger (1897) discovered 10 persons harbouring typical diphtheria bacilli out of 134 in an infected school (7·4 %). All the infected contacts shared the rooms of the diphtheria patients and were as a rule the next neighbours. Loeffler (1894) discovered virulent diphtheria bacilli in the throats of four out of 160 school children, examined at a time when diphtheria was prevalent (2·5 %). Pennington (1907) recently examined by means of cultures 375 apparently normal children from the public schools of Philadelphia. Diphtheria seems to have been prevalent in the city at the time. Typical diphtheria bacilli were found in the throats of 40 (9·3 %). Aaser (1895) during an outbreak in a cavalry regiment examined 89 well persons and found diphtheria bacilli, all of which were virulent, in 17 (19 %). Golowkoff (1898) examined 70 cadets during a small outbreak and found diphtheria bacilli in four (5·7 %).

(*e*) *Contacts not in the above classifications.*

Denny (1900) examined 50 contacts and discovered diphtheria bacilli in 6 (12 %). Meade Bolton (1896) amongst 214 persons who had been previously exposed to diphtheria found the virulent bacilli in 45·5 %, and Chapin (1902) found diphtheria bacilli in the throats of 16 % of persons exposed to the disease. Maude (quoted by Lack, 1899) found diphtheria bacilli in 89 (41 %) out of 214 exposed persons. Goadby (1900) obtained cultures from 586 children of the Poplar Union School in which 21 cases of diphtheria had previously occurred. He found in 190 cases (32·4 %) bacilli morphologically identical with diphtheria bacilli. This observation cannot be included amongst the others on school contacts since virulent diphtheria bacilli were found in the milk, and many of those showing diphtheria bacilli probably acquired them from the milk rather than by contact.

Table showing the results of investigations on the infection of healthy contacts with diphtheria bacilli.

Names of observers	No. of persons examined	No. of infected persons found	Percentage of infected persons	
Infected families.				
Cobbett (1901)	9	9	100	
Park (1892)	4	4	100	
Scheller (1905)	16	14	87·5	66 %
Spirig (1899)	9	6	66·6	
Williams (1896)	5	3	60	
Park and Beebe (1894)	48	24	50	

Table showing the results of investigations on the infection of healthy contacts with diphtheria bacilli (continued).

Names of observers	No. of persons examined	No. of infected persons found	Percentage of infected persons	
Persons attending on the sick.				
Richmond and Salter (1898)	129	62	48	37 %
Pugh (1902)	56	7	12·5	
Relatives of the sick.				
Scheller (1905)	284	108	38	29 %
Kober (1899)	128	15	11·7	
Hospital wards and Institutions.				
Lister (1898)	125	61	48	
Minnesota Board (1900) (Bethany Home)	242	69	28	
Johannessen (1895)	38	7	19·5	
Müller (1897)	90	14	16	
Soerensen (1898–9)	2187	274	12·5	14 %
Graham-Smith (1904)	48	6	12	
Park and Beebe (1895)	55	6	11	
Gross (1897)	314	24	7·6	
Minnesota Board (1900) (Faribault)	50	3	6	
Chatin and Lesieur (1900)	75	2	2·6	
Schools.				
Crowley and Eurich (1904)	93	42	45	
Peck (1901)	100	31	31	
Cobbett (x. 1901)	63	13	20	
Aaser (1895)	89	17	19	
Minnesota Board (1900)	225	30	13	
Berry and Washbourn (1900)	148	19	12	
Gabritschewsky (1901)	296	31	10	
Graham-Smith (1902)	519	54	10	8·7 %
Pennington (1907)	375	40	9	
Denny (1900)	190	16	9	
Thomas (1904)	1027	77	7·5	
Fibiger (1897)	134	10	7·4	
Golowkoff (1898)	70	4	5	
Graham-Smith (1904)	1018	42	4	
Cobbett (iv. 1901)	650	19	3	
Loeffler (1894)	160	4	2·5	
Other Contacts.				
Meade Bolton (1896)	214	97	45·5	36 %
Maude	214	89	41·5	
Denny (1900)	50	6	12·0	
Infection probably through both milk supply and contact.				
Goadby (1900)	586	190	32	
	9881	1453	14·7	

Summary.

From the statistics, which have been collected, it can be seen that the proportion of infected contacts depends to a great extent on their relationship to the diseased persons. Amongst the members of infected families in which no precautions are taken to isolate the sick, the proportion of infected contacts may be very high, whilst in families in which every precaution is taken the proportion may be as low as 10%. More distant relatives as well as persons attending on the sick may become infected in large numbers.

The observations on the inmates of hospital wards and institutions show that a considerable number of such persons are liable to become infected, when the disease breaks out amongst them. Amongst the scholars of infected schools the proportion of children who acquire diphtheria bacilli by contact is naturally smaller, since the opportunities for infection are not so great. Nevertheless, as some of the observations which are quoted show, a high proportion may become infected if little check is placed on the spread of the disease.

The statistics quoted on close contacts, namely members of infected families, relatives, and attendants, show that amongst such persons 36·6% are liable to become infected, while the mean infection amongst inmates of hospital wards and institutions is 14%, and amongst scholars of infected schools 8·7%.

It must be remembered, however, that in these statistics several factors have not been taken into account, which have a great influence on the results. Amongst these are the regulations which are in force for isolating the sick, as soon as they develop the disease, and for keeping them isolated until the diphtheria bacilli have disappeared from their throats and noses. The number of mild unrecognised cases, which are not isolated and go about spreading infection, also exerts a great influence. Again in hospital wards and schools it is generally found that those most exposed to the sick persons by being placed near them for considerable periods are the individuals amongst whom the proportion of infection is highest, so that when these persons only are examined the proportion of infected contacts amongst these classes is raised.

The investigations on the virulence of diphtheriia bacilli in contacts are given at length in Chapter VI, where it is shown that a very large proportion of these persons harbours fully virulent bacilli. In two classes (*c* and *d*) the inoculation experiments which have been carried out showed that 66% and 81% respectively of the strains tested were virulent.

III. The distribution of diphtheria bacilli amongst healthy persons, who have had no opportunity of acquiring them by contact.

Throughout the literature on diphtheria statements are frequently to be met with which indicate that the authors consider that virulent diphtheria bacilli may be encountered in a certain proportion of healthy individuals, who have not recently been exposed to persons suffering from the disease, or to infected contacts.

Reliable observations on this point, however, are very few, since many of the investigators have not gone to the trouble of isolating and testing the organisms which they have found, but have based their diagnoses on the morphology of the bacilli in culture; nor have they usually made special inquiries into the possibility of recent contact.

Observations of this kind can only indicate in what proportion of persons organisms, more or less resembling diphtheria bacilli, occur, but no conclusions as to their power of transmitting the disease can be made. Nevertheless some observers have drawn far-reaching conclusions from the results of these experiments, even to the extent of stating that one out of every seven normal children amongst the community harbour diphtheria bacilli in their throats, and are therefore a possible source of danger.

In considering these observations, therefore, those which rely for the most part on the morphology in culture without reference to the possibility of recent contact, are separated from those which are based on a thorough examination of the organisms found, including the test on animals, together with inquiries into the antecedents of the infected persons.

(*a*) *The following statistics are for the most part based on observations relying on the morphology in culture alone.*

Baurowicz (1895) examined the nasal secretion in 50 persons, 40 of whom were suffering from various diseases, but found no diphtheria bacilli.

Besser (1889) also examined the nasal secretion of 57 normal persons without finding diphtheria bacilli.

Beck (1890) made observations on the throats of 66 healthy children and 64 suffering from various diseases without finding diphtheria bacilli, and Zarniko (1890) examined with the same results 18 throats of normal, and 22 of diseased, persons.

Neumann (1902) found no diphtheria bacilli in the noses of 111 normal persons, but found one amongst 87 persons suffering from non-diphtheritic nasal diseases.

Goadby (1900) examined 100 children in a school in which there had been no clinical cases of diphtheria for two years, and found 18 children harbouring diphtheria bacilli, of whom 14 had unhealthy throats. Diphtheria, however, seems to have been prevalent in the neighbourhood.

Parkes (1903) made cultures from 814 children admitted into the Chelsea Hospital and found diphtheria bacilli in 88, and Hewlett and Murray (1901) on examining 385 children admitted into the Victoria Hospital found diphtheria bacilli in 58.

Park and Beebe (1895) considered that at the time of their examinations virulent diphtheria bacilli were present in 1% of the healthy throats in New York. They remark, however, that diphtheria was rather prevalent at the time, and express the belief that "most of the persons in whose throats they exist have been in direct contact with cases of diphtheria."

The Committee of the Massachusetts Board of Health (1902), in their "Report on diphtheria bacilli in well persons," give tables showing the results of the examination of 4250 well persons, mostly from institutions and schools. These observations are almost entirely confined to the morphology of the bacilli in culture. The results in detail are given in the following table:—

Place examined	No. of persons examined	No. of persons infected	Percentage of persons infected	Remarks
Ontario	50	0	0	Patients and attendants in general hospital.
Newton	63	0	0	Women students in Wellesley College.
Springfield	185	0	0	64 school children, 121 prisoners.
Providence	927	4	0·43	541 school children, 376 tramps, 10 smallpox patients.
Waltham	297	2	0·67	
Lowell	250	2	0·8	Cotton-mill hands.
Washington	221	2	0·9	Mostly hospital patients.
Boston	892	27	3·0	Adults in prison and pauper institutions.
Orphan Asylum	65	2	3·0	
Brookline	129	3	2·3	School children — All these persons were more or less exposed to diphtheria.
Willard Hospital	82	3	3·6	,, ,,
Owatonna	247	13	5·2	,, ,,
Park Rapids	316	17	5·3	Children and adults
Red Wing	382	22	5·7	,, ,,
Old Ladies Home	42	5	11·9	,, ,,
Bethany Hospital	102	21	20·5	,, ,,

Amongst these 4250 persons 2·89 % harboured morphologically typical diphtheria bacilli. It can be seen, however, that the last seven sets ought more properly to be placed amongst contacts. Excluding these sets 2955 persons, apparently non-contacts, were examined and 39 (1·3 %) showed diphtheria bacilli in their throats. Of these 39 strains of morphologically typical diphtheria bacilli, 12 were tested on animals, and all proved to be non-virulent. Seventeen strains of uniformly staining bacilli were also tested, and three proved to be virulent, all of which were obtained from Providence. Consequently no morphologically typical *virulent* bacilli were found amongst 2028 of these non-exposed persons from eight different places, whilst amongst the remaining 927 from Providence virulent but atypical diphtheria bacilli were found in 0·3 %.

Even if the persons more or less exposed to diphtheria are included, the proportion of virulent bacilli observed is found to be very small. Altogether 4250 persons, exposed and non-exposed, were examined, and in the throats or noses of 123 morphologically typical diphtheria bacilli were discovered (*A*, *C*, and *D* types of Wesbrook). Of these strains 41 were tested on animals and two (5 %) proved to be virulent (Brookline), and 39 (95 %) non-virulent. Of 30 uniformly staining types 27 were non-virulent and three virulent (Providence). One barred form was also examined and found to be non-virulent. Only 7 % therefore of all the bacilli tested were found to be virulent. If the proportion of virulent forms was the same amongst the strains which were not tested only ·14 % of all these persons harboured virulent diphtheria bacilli.

In considering the results of Hewlett and Murray and of Parkes, the results of the Massachusetts Committee and the conclusions of Pennington (1907) and of Pugh (1902) must be borne in mind. The latter observer considers that "in large centres of population, where diphtheria always exists, diphtheria bacilli are to be found in a not inconsiderable proportion of school children. In the absence both of the evidence of clinical diphtheria and of a history of exposure to that affection, the bacilli are, in the majority of cases, of a non-virulent or saprophytic type and of little hygienic importance; in cases on the other hand, where the clinical supports the bacteriological examination, the bacilli are almost certainly virulent, and therefore dangerous; while in cases where the patient is known to have been exposed to infection the chances are great that the organisms are of the pathogenic variety, and such cases should always be regarded with grave suspicion." The

Massachusetts Committee (1902, p. 21) also came to a similar conclusion. They say "that if a healthy person is found to have Klebs-Loeffler bacilli, and there is no connection traceable between that person and a case of diphtheria, the chances are very much in favour of the bacilli being non-virulent." Pennington (1907), as the result of a considerable number of inoculation experiments, comes to the conclusion "that the organisms found in the throat of a well person are, in the majority of cases, without virulence."

Hewlett and Murray only examined three cultures for virulence, and found that while two were non-virulent the other was only slightly virulent.

The following table shows the prevalence of diphtheria bacilli amongst normal persons and others suffering from non-diphtheritic diseases, as ascertained by investigations in which few inquiries appear to have been instituted as to the possibility of recent contact, and in which the virulence of the organisms found was seldom tested:

Observer	Persons examined	Morphologically typical diphtheria bacilli	Bacilli tested for virulence	Virulent diphtheria bacilli	Non-virulent diphtheria bacilli
Massachusetts Committee[1] (1902)	2955	39	12	0	12
Hewlett and Murray (1901)	385	58	3	1	2
Parkes (1903)	814	88	0	—	—
Goadby (1903)	100	18	0	—	—
Neumann (1902)	198	1	0?	—	—
Baurowicz (1895)	50	0	0	—	—
Beck (1890)	130	0	0	—	—
Zarniko (1890)	40	0	0	—	—
Besser (1889)	57	0	0	—	—
	4729	204 (4·3 %)	15	1	14

This series of observations shows that organisms morphologically resembling diphtheria bacilli in all respects are to be found in the mouths and noses of a small proportion of the normal population. The few inoculation experiments which have been carried out tend to prove, however, that the great majority of these bacilli are non-virulent, and therefore probably incapable of giving rise to diphtheria in man.

[1] In the table the more or less exposed persons have been omitted. The virulence tests on those organisms which the Committee did not consider typical have also been omitted.

(*b*) *The following statistics only include those in which the suspicious organisms have been tested and in which careful inquiry was made as to the possibility of recent contact.*

Kober (1899) examined 600 healthy school children from 14 different classes, and discovered diphtheria bacilli in 15. Of these 15 twelve had fairly recently come in contact with the disease, and five of them harboured virulent bacilli. The remaining three, from whom no history of contact was obtainable, showed non-virulent bacilli (non-contacts with virulent bacilli 0 %).

Denny (1900) examined 235 healthy individuals (216 children and 19 adults), a large proportion of the well-to-do classes. He only once, in a school girl, found the diphtheria bacillus. So far as was known the girl had not been in contact with a case of diphtheria, and the bacilli were so few that a pure culture could not be obtained (·4 %).

Cobbett (1901) examined 43 healthy school children, and found no diphtheria bacilli. In some recent investigations, which have not yet been published, this author examined cultures from the throats of 91 boys in a reformatory school, isolating and testing for virulence all the diphtheroid organisms, typical and atypical, which were found. Only in the case of a boy who had just joined the school were diphtheria bacilli found. The other 90 had not been in contact with the disease and no diphtheria bacilli were found in their throats (see also p. 212).

Park and Beebe (1895) examined 275 persons, chiefly hospital patients. Diphtheria bacilli were obtained in pure culture from 26 of them, but 23 cultures proved to be non-virulent. The presence of one of the three virulent examples was accounted for by recent contact, and the other two occurred in adults attending the New York Dispensary at a time when diphtheria was prevalent in the city.

Pugh (1902, p. 296) examined the throats of 415 unselected scarlet fever patients admitted into the North-Eastern Fever Hospital, who showed no clinical signs of diphtheria. In the throats of 17 diphtheria bacilli were found. Of these strains five were inoculated into animals and found to be completely non-virulent, and since the others occurred under the same conditions Pugh assumed that they were non-virulent.

Graham-Smith (1903) made cultivations from the throats of 362 healthy non-contacts, and in one case found non-virulent diphtheria bacilli.

Pennington[1] (1907) examined 125 school children from non-infected schools in Philadelphia. Thirteen harbouring diphtheria bacilli were found. Of these strains 11 (86·4 %) were non-virulent, and two virulent. In the latter the evidence excluding the possibility of contact is not very satisfactory.

In another set of investigations 16 strains were obtained from non-contacts of which 11 (69 %) were non-virulent. As diphtheria was prevalent at the time the pathogenic strains may have been derived by contact.

The following table shows the results of observations on normal throats in which careful inquiries were instituted as to the possibility of recent infection, and in which the majority of the suspicious organisms were isolated and tested for virulence.

Observer	No. of non-exposed persons examined	Persons harbouring virulent diphtheria bacilli	Persons harbouring non-virulent diphtheria bacilli
Park and Beebe (1895)	274	2	23
Kober (1899)	588	0	3
Denny (1900)	235	0	1?
Cobbett (1901)	43	0	0
Cobbett (unpublished)	90	0	0
Pugh (1902)	415	0	17
Graham-Smith (1903)	362	0	1
Pennington (1907)	125	2	11
	2132	4 (·18 %)	56 (2·62 %)

Persons found on inquiry to be recent contacts have been excluded from this table.

Remembering the great difficulty often met with in prosecuting inquiry amongst school children, and the class of persons from whom hospital cases are drawn, and amongst whom these investigations were principally conducted, these figures are very striking, and in the absence of further evidence undoubtedly point to the conclusion that virulent diphtheria bacilli are seldom, if ever, present in the throats of healthy persons who have not recently been in contact with cases of diphtheria or infected contacts.

[1] This observer usually inoculated at least two guinea-pigs with bacilli obtained from different cultures from each child, making use of bacilli scraped from the surface of the serum. He gives very full tables relating to the possibility of contact in each case. The figures here given are derived from these tables omitting two or three cases in which the data are insufficient.

The Distribution of Non-virulent Diphtheria Bacilli amongst Contacts and Non-contacts.

In the last section it has been shown that non-virulent diphtheria bacilli are to be found in the throats of about 2·6% of healthy individuals, who have not been exposed to the disease. The observations of Pugh (1902) and of the Massachusetts Committee (1902) show that they are also to be found in the noses of such persons. The former obtained from the nasal cavities of 414 scarlet fever patients on admission to hospital, who showed no evidence of faucial diphtheria or fibrinous rhinitis, 33 bacilli morphologically indistinguishable from the diphtheria bacillus. Of these six cultures were tested on guinea-pigs and all proved to be non-virulent.

Observations relating to the occurrence of non-virulent diphtheria bacilli amongst contacts are not very numerous. Cobbett (IV. 1901) tested the virulence of several strains of diphtheria bacilli which he obtained from healthy contacts. In one outbreak he found 19 out of 650 persons, who had been more or less exposed to the disease, to be harbouring diphtheria bacilli. Nine cultures were tested for virulence, and three were found to be non-virulent. In another outbreak (X. 1901) he examined 63 children from an infected school, and found diphtheria bacilli in ten. Of these ten strains four were non-virulent. At least seven persons therefore (0·9%) out of 713 harboured non-virulent diphtheria bacilli.

Amongst 1200 persons, more or less exposed to diphtheria, and who were not harbouring virulent diphtheria bacilli, the writer (Graham-Smith, 1904) found 15 who showed non-virulent organisms, morphologically and culturally indistinguishable from diphtheria bacilli (1·2%). Pennington (1907) found 19 (5·7%) non-virulent strains on cultures from the throats of 375 school children, most of whom were attending infected schools. In these three sets of observations nearly 2300 exposed persons were examined, and at least 41 harbouring non-virulent diphtheria bacilli discovered (1·7%).

It would appear, therefore, that in the mouths of 1—2% of healthy persons, whether recently exposed to diphtheria or not, non-virulent diphtheria bacilli are to be encountered. These figures lend some support to the view, which has been occasionally advanced, that some at least of the so-called non-virulent diphtheria bacilli belong to a separate saprophytic species, though at present they cannot be distinguished by morphology or culture from true virulent diphtheria bacilli.

The Distribution of Diphtheria Bacilli in the Noses of Healthy Persons.

It is undoubtedly the case that a certain proportion of persons may become infected in their noses and not in their throats. In examinations of contacts conducted on the throat only, such persons would be missed. The observations on this point are not very numerous, and the principal records are tabulated below.

Table showing the proportion of persons infected with morphologically typical diphtheria bacilli in the nose, the throat being free.

Observer	No. of persons examined	Persons infected in the nose only	Percentage	
Contacts. (*Hospital wards and Institutions.*)				
Gross (1897)	314	17	5·3	
Minnesota Board (1900)				
Bethany Home	242	19	7·6	
Faribault	50	1	2·0	
Massachusetts Committee (1902)				6·3 %
Willard State Hospital	82	2	2·4	
Bethany Hospital	102	11	10·7	
Old Ladies Home	42	3	7·1	
Schools.				
Minnesota Board				
Owatonna	40	1	2·5	
Mankato	30	0	0	
Albert Lee	24	0	0	
Faribault	57	0	0	
Massachusetts Committee				1·9 %
Brookline	129	1	0·7	
Owatonna	247	6	2·0	
Red Wing	382	5	1·3	
Park Rapids	316	11	3·5	
Non-Contacts.				
[1] Besser (1889)	57	0	0	
[1] Baurowicz (1895)	50	0	0	
[1] Lack (1899)	100	13	13	
[1] Neumann (1902)	198	1	0·5	
Pugh (1902)	414	23	5·5	
Massachusetts Committee				
Ontario	50	0	0	
Newton	63	0	0	1·5 %
Springfield	185	0	0	
Washington	221	0	0	
Lowell	250	2	0·8	
Waltham	297	2	0·6	
Providence	927	4	0·4	
Boston	892	13	1·5	
Orphan Asylum	65	1	1·5	
	5,826	136	2·33	

[1] Simultaneous cultures from the throat were not made in these examinations.

In regard to these observations the virulence experiments of the Massachusetts Committee have already been quoted. Pugh's experiments are of especial interest in that his observations were made on unselected cases of scarlet fever on admission into hospital, who showed no clinical evidence of diphtheria. Three of his 23 strains of bacilli were inoculated into guinea-pigs, and all were found to be non-virulent.

Conclusions on the Distribution of Diphtheria Bacilli.

The various records which have been quoted in the preceding pages show that only about 71 % of the persons notified as suffering from diphtheria show any bacteriological evidence of the disease. The proportion of those healthy persons who become infected with diphtheria bacilli by contact with patients, and yet do not suffer from diphtheria, depends largely on the intimacy of their connection with the patients, varying from a mean of 66 % in members of the family, if the conditions for spreading are favourable, to a mean of 8·7 % in the less closely connected scholars in infected schools. The great majority of the bacilli which have been isolated from these contacts and tested on animals have been found to be virulent (see p. 231).

In the throats and noses of healthy persons, who have had no opportunity of acquiring them by contact, virulent diphtheria bacilli are very rarely found, but bacilli resembling them in morphology, but differing in being totally without virulence for guinea-pigs, are more common (2·6 %). Even amongst recently exposed persons such organisms are to be encountered in a small percentage (1·7 %).

Summary of Chapter IV.

The diphtheria bacillus, as obtained from young cultures on serum, is met with in several morphological types, differing mainly in staining peculiarities, but resembling each other in their general features. Long, medium, and short forms are found. None of these types are absolutely peculiar to the diphtheria bacillus, since organisms indistinguishable in morphology have been reported from various sources. The majority of diphtheria bacilli from young serum cultures show polar bodies. The presence of polar bodies does not indicate virulence, nor does their absence indicate a lack of virulence. Similar polar bodies have been found in several other organisms. So far as the investigations on the subject have been carried the "snapping" type of fission movement

is peculiar to the diphtheria group of organisms. Many cultures of diphtheria bacilli show branching forms. Diphtheria bacilli are non-motile, and do not form spores under any known conditions. Cultures of diphtheria bacilli do not show any specific characters by which they can be separated from all other diphtheroid organisms. The most important characters are the production of a marked acid reaction in media to which glucose has been added, an invisible growth on potato, and the formation of a granular deposit in broth. The growths obtained on serum and agar are also helpful in diagnosis. The morphological and cultural characters therefore of the diphtheria bacillus, while of great service in distinguishing it from other diphtheroid organisms, are none of them absolutely characteristic. The diphtheria bacillus does, however, differ from all other known organisms in producing a specific toxin, whose action on animals can be neutralised by antitoxin. The bacilli themselves, or their products, when injected subcutaneously into guinea-pigs give rise to specific lesions. Bacilli, indistinguishable from true virulent diphtheria bacilli, occur, which are entirely non-pathogenic to laboratory animals. They are commonly regarded as diphtheria bacilli which have for some reason lost their virulence, and are termed attenuated or non-virulent diphtheria bacilli. Between the fully virulent and the totally non-virulent diphtheria bacilli races of varying degrees of virulence have been isolated, but the occurrence of such races is probably much rarer than has commonly been supposed.

Diphtheria bacilli in a moist condition are killed by an exposure to a temperature of 58° C. for ten minutes, but in a dried condition, or when enclosed within membrane, they exhibit considerable powers of resistance. They are capable of multiplying very rapidly in raw milk.

Diphtheria bacilli are found in about 71 % of the cases clinically diagnosed as diphtheria, and occur in a varying percentage of persons who have been exposed to the disease, depending on the intimacy of their relation to the sick. Although non-virulent diphtheria bacilli are occasionally found in the throats and noses of persons who have not recently been exposed to the disease, virulent diphtheria bacilli are rarely or ever encountered under such conditions.

Note.—Sholley (15. VI. 07) has recently recorded observations on 1000 children, mostly living in tenement houses, who came for treatment to the New York hospitals. "As far as possible inquiry into immediate and recent exposure was made and noted, and no child suffering from an angina or anything suggesting a sore throat, nasal discharge or laryngitis, was included in the series. Eighteen showed virulent diphtheria bacilli (1·8 %) and 38 non-virulent (3·8 %).

CHAPTER V.

THE PSEUDO-DIPHTHERIA OR HOFMANN'S BACILLUS.

Morphology. Reaction to staining agents. Involution forms. Appearances at different stages of growth. Post-fission movements. Branching forms. Influence of temperature. Cultivation on artificial media, serum, agar, gelatin, potato, broth, milk, litmus whey. Indol production. Virulence. Distribution. Seasonal prevalence. Summary.

The Pseudo-diphtheria or Hofmann's bacillus.

It has been already briefly pointed out that the term pseudo-diphtheria bacillus has been made use of by various writers to include several species of bacilli. Some have included under this term all diphtheria-like bacilli differing in any respect from typical diphtheria bacilli. Others again seem to have restricted it to the non-virulent diphtheria bacillus. Finally, many writers have used it to indicate the short, non-pathogenic, non-acid forming bacillus of Hofmann. In this and the following chapters the term will only be used in the latter sense.

The bacillus of Hofmann, or pseudodiphtheria bacillus, is frequently found in the healthy and diseased buccal and nasal secretions.

The Morphology of Hofmann's bacillus.

A. In *smear* preparations from the buccal and nasal secretions the morphological characters of Hofmann's bacillus are much the same as in preparations made from serum cultures, but the organisms are found lying scattered over the field without any special relation to one another. They are occasionally found enclosed within cells.

B. *In culture. Arrangement.* Preparations made from cultures grown on Loeffler's or other serum media show the bacilli usually arranged in small groups of several individuals generally lying parallel

to each other. Similar lines of parallel bacilli are frequently found close to each other, but disposed at various angles. Separate individuals or small clumps without any parallel arrangement are not infrequently seen. According to the experience of the writer, the parallel grouping just described is particularly characteristic of this organism. Chains are never formed (Pl. XV, fig. 2).

Size. This organism is generally much shorter than the common forms of the diphtheria bacillus, but forms as long as medium length diphtheria bacilli are occasionally present even in young cultures. The length of typical examples of Hofmann's bacillus varies between 1—1·5 μ.

Shape. The typical Hofmann's bacillus appears as a short, straight, oval bacillus, with rounded ends, and one median, lightly stained transverse septum. In proportion to its length it is much broader than the diphtheria bacillus, and the widest part, which is about equal to 1/8 of the length, is at the middle. Colonies are not infrequently found in which the bacilli are rather longer, and relatively narrower than those described, but resemble them in all other respects.

A few individuals are occasionally met with even in young colonies which are longer than the ordinary forms and have more than one unstained septum, and may have several. Some colonies have a fair number of such individuals (Cobbett, 1901, p. 239), and more rarely colonies are encountered in which nearly all the bacilli are of this form (Graham-Smith, 1903, p. 230). Some of these forms are clubbed and often slightly curved, but are broader and take the stain more deeply than diphtheria bacilli. The stained segments are very dark and well defined and the septa are narrow and run in all cases transversely across the bacilli (Pl. XV, fig. 1). In subcultures such forms revert to the typical short form of Hofmann's bacillus, and behave like it in all cultural characters[1]. In cultures of typical Hofmann's bacilli over 24 hours old the proportion of segmented and long forms is frequently, but not always, increased. Sometimes after 48 hours' growth the bacilli are even shorter than they were at an earlier period.

Staining of the protoplasm. With Loeffler's methylene blue (full strength or diluted 1 : 5), these bacilli stain darkly and, with the

[1] On serum media the longer forms are only to be met with in cultures taken direct from the throat, when the morphological characters may possibly be influenced by the presence of other organisms or of mucus. *Young* pure cultures from all such forms show only the oval type.

exception of the medium septum, evenly. The septum or, in the case of the long forms, the septa are only very slightly stained in the majority of cases. Sometimes bacilli are found which stain uniformly without a median light septum. When mounted by Cobbett's method (see p. 144) in dilute methylene blue solution, almost entirely unstained specimens, like shadows of bacilli, are always to be seen amongst the darkly stained specimens. In young cultures metachromatic granules (polar bodies) are rarely encountered in any of the forms, but occasionally a bacillus may show one or two very small polar bodies, and very rarely a considerable proportion of the bacilli may do so.

In cultures 36 or more hours old polar bodies are occasionally found, especially in the enlarged forms. These, however, are seldom as sharply defined as in diphtheria bacilli, and are frequently ovoid or elongated instead of round. Stained by Neisser's and other special methods the bacilli in young cultures show no polar bodies except under the conditions just mentioned. The capsules may be demonstrated by Boni's (1900) method.

Involution Forms. Some old cultures of Hofmann's bacillus show numerous and well marked involution forms, others do not. In some cultures, even after prolonged cultivation in broth or on agar, the majority of the bacilli remain short, though they may show more than one light band, and very few long forms are to be found. In other cultures, however, a large number of long and enlarged bacilli may occur under these conditions. Some of the long forms resemble those described as occasionally occurring on young serum cultures, other giant forms may be very long, thick, curved, clubbed and segmented, others again may be oval or pear-shaped. In fact some old cultures of Hofmann's bacillus so closely resemble old cultures of the diphtheria bacillus as to be scarcely distinguishable.

Morphological appearances of the Hofmann's bacillus at various periods of growth on serum.

Denny (1903) studied cultures on serum tubes incubated at 36° C. by means of stained preparations made after 4, 8, 12, 15, 24, 36, and 48 hours' growth. He found that no changes in shape and size took place up to 36 hours, but sometimes after 48 hours' growth the bacilli appeared even shorter than they were at first (p. 132, Pl. IX, figs. 5, 6).

Post-fission movements.

Hill and Rickards (1903) studied the method of division in Hofmann's bacilli, and stated that "snapping" occurred as in diphtheria bacilli and that the post-fission movements were very similar.

Branching forms.

Abbott and Gildersleeve (1903) studied a number of cultures, but never observed branching even when the method most favourable to its exhibition by genuine diphtheria bacilli was employed, nor have branching forms been described by any other authors.

The influence of temperature in relation to the growth of Hofmann's bacillus on artificial media.

Hofmann's bacillus begins to develop at a lower temperature (18° C.) than the true diphtheria bacillus, and growth is more luxuriant. In other respects the conditions of temperature affecting its growth are the same as in the case of the diphtheria bacillus.

The thermal death point in liquid media is 58° C. for ten minutes.

Cultivation on artificial media.

Hofmann's bacillus is *non-motile, without flagella,* and does *not produce spores* under any known conditions.

Loeffler's serum. Hofmann's bacillus grows very rapidly on Loeffler's serum at 36—37° C. After 18—24 hours' incubation the colonies are small, rounded, elevated, and usually pure milk-white in colour. They have a shiny, moist surface and a distinct margin, and are usually discrete, though in crowded cultures the colonies show some tendency to run together. As compared with those of the diphtheria bacillus, the colonies are much whiter, softer, and larger, and usually flatter. Indentation of the margin or radial fissuring can rarely be seen even with the aid of a simple lens. Growth between 24 and 48 hours is usually much more rapid than that of the diphtheria bacillus, the colonies attaining considerable dimensions during this period. In old cultures similar changes take place in the colonies as in the case of the diphtheria bacillus, they become larger and flatter and lose their shining appearance and concentric rings and radial striae can be seen on the surface. At the same time the margins become irregular, and sometimes even daisy-shaped colonies are produced.

On alkaline serum the colonies in the early stages resemble closely those of the diphtheria bacillus, but are usually larger, and somewhat whiter. At a later period they can usually be distinguished by their whiter colour and larger size. Their general characters on this medium are the same as on Loeffler's serum.

The *morphological characters* on these media have already been described.

Nutrient Agar and Glycerine Agar. At 37° C. Hofmann's bacillus grows more luxuriantly than the diphtheria bacillus on agar, forming in 24—48 hours large white, shining, moist, round, smooth, raised colonies with distinct margins without indentations. The colonies retain these characters during several days' incubation, but gradually lose their moist and shiny appearance, and later become flatter and somewhat depressed in the centre. In crowded cultures there is some tendency for the colonies to coalesce.

In *stab* cultures Hofmann's bacillus produces a more luxurious growth than the diphtheria bacillus both on the surface and along the needle tract.

Morphology. In the earlier stages of growth short oval forms only are found, but as growth progresses segmented forms become fairly common, and after a few days numerous involution forms may be seen.

Gelatin. The colonies, produced after some days' growth on gelatin, are very similar to those formed upon serum; namely whitish, circular colonies, well separated from each other. Their surfaces are uniformly rounded and show little or no tendency to indentation of the margin or radial striation. When grown upon gelatin to which 1 % of glucose has been added they assume a more brilliant white colour, and show a tendency to become irregularly spiked at the margin. (Cobbett and Phillips, 1896.) Liquefaction does not occur.

Hofmann's bacillus grows more luxuriantly on this medium than does the diphtheria bacillus. In *stab* cultures *growth* occurs both on the surface and along the needle tract.

Morphologically the bacilli resemble those from serum cultures.

Potato. On potato a copious but invisible growth occurs like that of the diphtheria bacillus. In cover-glass preparations made from early potato cultures the oval form of the bacillus is found, but in older cultures giant, segmented, occasionally clubbed, forms are not rare.

Broth. In ordinary broth incubated at 37° C. a good growth occurs somewhat similar to that of the diphtheria bacillus, but early diffuse clouding of the medium is more common. The tendency, as with the latter

organism, is for the cloud to become gradually deposited as fine granules on the sides and bottom of the test tube. Not infrequently a slight surface film may be formed. The reaction of the broth tends to become more alkaline and acid is never produced.

Morphology. In broth cultures this bacillus more closely resembles the diphtheria bacillus than on other media. Here too the oval bacillus with one unstained septum predominates, but giant forms which closely resemble diphtheria bacilli are generally present also, and may be numerous. They are many times the length of the oval bacillus, often clubbed, and are rather thicker than the diphtheria bacillus.

In *glucose broth* the growth is similar to that produced in ordinary broth, but the clouding is often more marked. Neither gas nor acid is ever produced, and in fact there seems to be even an increase in alkalinity.

Sugars and carbohydrates. Hofmann's bacillus does not produce acid even after prolonged growth in broth or Hiss's medium containing glucose, lactose, maltose, galactose, saccharose, laevulose, dextrine, mannite, or glycerine. (Knapp 1904, Benham 1906, Hamilton and Horton 1906, Graham-Smith 1906.)

In *milk* prepared as a culture medium a good growth occurs. The medium does not become acid, nor is it coagulated. Abundant growth occurs in *litmus whey*, but the medium remains alkaline.

Indol. According to Hewlett (1901) old cultures of the Hofmann's bacillus, like those of the diphtheria bacillus, produce skatol-carbolyxic acid, which has been mistaken for indol (see p. 164).

Anaerobic cultures. According to Hewlett and Knight (1897, p. 14) Hofmann's bacillus does not grow anaerobically in hydrogen. Most other writers, who mention the subject, state that growth occurs under the ordinary conditions of anaerobic cultures.

Powers of resistance.

No experiments appear to have been made on the powers of resistance of Hofmann's bacillus to drying, cold, putrefaction, sunlight or disinfectants, or on its capacity for growth on various food substances.

Virulence.

Towards the ordinary laboratory animals, guinea-pigs, rabbits, rats, etc., Hofmann's bacillus is not pathogenic, when injected subcutaneously or intraperitoneally even in large doses. With very large doses a slight

and transient oedema may be the result. According to some observers, however (Williams, 1902), guinea-pigs may succumb to the intraperitoneal injection of enormous doses.

Some writers have asserted that this bacillus is pathogenic for small birds of the finch and bunting families. (For an account of these experiments see p. 220.)

The possibility of causing Hofmann's bacillus to become virulent to guinea-pigs in small doses, its action on small birds, its power of producing toxoids in culture, and its agglutination by the sera of animals immunised to the diphtheria bacillus, are questions which are intimately bound up with its relationship to the diphtheria bacillus, and are discussed in the next chapter.

The distribution of Hofmann's bacillus.

Nearly all observers are agreed that the Hofmann's bacillus is to be met with in greater or lesser numbers in the throats and noses of persons recovering from diphtheria, and also of healthy persons. While some consider that they are more frequently discovered in the former class and contacts, others think that they are as common in healthy non-contacts, as in convalescents and contacts. The statistics on the subject are, however, extremely conflicting, partly owing to diverse opinions as to which organisms should be considered as pseudo-diphtheria or Hofmann's bacilli, and partly to the different methods which have been adopted. Some investigators on the one hand have founded their impressions on observations dealing with certain limited classes of the community only, and on the other hand it is not uncommon to find statistics based on experiments which include without distinction members of the following classes: adults and children, non-contacts and contacts, healthy persons and those suffering from diseases of the nose and throat, persons chosen at random and those living under peculiar conditions in schools, institutions, asylums, and hospital wards. Again in very few cases has any attempt been made to separate persons belonging to the well-to-do classes from those belonging to the poorer classes.

In the first of the following tables a considerable number of observations have been brought together, regardless of any of the above considerations, showing the percentage of healthy persons found to be infected in either the throat or nose, or both, with Hofmann's bacillus. There is also added the percentage in each case of persons infected with diphtheria bacilli. The table shows that at least 32 % of all these

persons were infected with Hofmann's bacillus, and that the number of those infected with this organism bears no relation to the number infected with the diphtheria bacillus.

Table (A) showing the total infection with Hofmann's bacillus in the throat, or nose, or both, of contacts and non-contacts, adults and children.

American observers	Number examined	No. infected with Hofmann's bacillus	Percentage infected with Hofmann's bacillus	Percentage infected with the diphtheria bacillus
Park and Beebe (1895)	330	27	8·1	9·7
Minnesota Board (1900)	443	128	28·8	20·1
Massachusetts Committee (1902)[1]				
(1) In the East	3096	562	18·1	1·5
(2) In Minnesota	1154	510	44·2	6·03
	5023	1227	24·4	
European observers				
Neumann (1902)	206	200	97·0	0
Pakes (1900)	441	428	97·0	0
Jacob (1905)	113	77	68	5·3
Hutchens (1906)	87	49	57	
Pugh (1902)	420	234	55·7	15·9
Lack (1899)	100	52	52	13·0
Graham-Smith (1902–4)	2878	1346	46·7	7·2
Cobbett (unpublished)	90	39	43·3	
Roux and Yersin (1890)	123	49	39	
Cobbett (1904)[2]	1724	673	39	
Chatin and Lesieur (1900)	75	22	30	2·6
Hasslauer (1902)	82	23	28	0
Thomas (1904)	1027	277	27	7·5
Hewlett and Murray (1901)	385	92	23·9	15
Berry and Washbourn (1900)	142	33	23	11·5
Parkes (1903)	814	162	19·9	10·9
Golowkoff (1898)	70	8	11·5	5·7
Symes (1895)	82	12	14·6	14·6
Fibiger (1895)	135	15	11·1	12
Stein (1900)	86	7	8·1	0
Sellner (1897)	103	7	6·8	0
Escherich (1895)	320	13	4·0	4
Garratt and Washbourn (1899)	666	21	3·2	
Hewlett and Thomson (1895)	103	0	0	0
Peters (1896)	25	0	0	0
Baurowicz (1895)	40	0	0	0
	10337	3839	36·9	
Total	15360	5066	32·9	

[1] Only type D^2 counted. Examples of Hofmann's bacillus were probably included under types E^2 and G^2, of which many occurred. If the latter types are all included the total infection in American observations is 38·9 %.

[2] Quoted by Graham-Smith (1903, p. 249).

In the following tables (*B* and *C*) an attempt has been made to subdivide the figures given in the previous table for the purpose of gaining a more minute estimate of the numbers of persons infected with Hofmann's bacillus under various conditions.

Table (B) showing throat infection amongst healthy persons with Hofmann's bacillus.

American observers	No. of persons examined	No. infected with Hofmann's bacillus	Percentage infected	
Adult non-contacts.				
Massachusetts Committee (1902)[1]				
Ontario	50	3	6·0	
Boston	892	45	5·0	
Lowell	250	0	0	5·5 %
Springfield[2]	185	15	8·1	
Park and Beebe (1895)	257	27	10·5	
Adult contacts.				
(M. C.) Willard State Hospital	82	0	0	
Children non-contacts.				
(M. C.) Washington	221	1	0·4	
,, Providence[3]	927	10	1·0	0·9 %
Park and Beebe (1895)	18	0	0	
Children contacts.				
(M. C.) Minnesota	1154	168	14·5	
,, Brookline	129	15	11·6	13·2 %
Minnesota Board of Health (1900)[4]	392	39	9·9	
	4557	323	7·0	
European observers				
Adult non-contacts.				
Escherich (1893)	320	13	4	
Adult contacts.				
Golowkoff (1898)	95	14	14·7	12·6 %
Fibiger (1895)	135	15	11·1	
Children non-contacts.				
Hutchens (1906)	87	49	57	
Roux and Yersin (1890)	59	26	44	
Cobbett (unpublished. See p. 212)	90	39	43·3	
Beck (1890)	107	36	33·6	24·1 %
Hewlett and Murray (1901)	385	92	23·9	
Parkes (1903)	725	162	22·3	
Pugh (1902)	420	67	15·9	

[1] Only type D_2 included.

[2] Springfield total made up of 121 male prisoners and 64 school children.

[3] Providence total made up of 386 adults and 541 school children.

[4] Total at Albert Lee, Faribault, Owatonna, and Mankato schools, and the Bethany Home.

M. C. = Massachusetts Committee (1902).

Table (B) showing throat infection amongst healthy persons with Hofmann's bacillus (continued).

European observers	No. of persons examined	No. infected with Hofmann's bacillus	Percentage infected	
Children contacts.				
Cobbett (1904)[1]	1724	673	39	
Roux and Yersin (1890)	45	15	33	
Chatin and Lesieur (1900)	75	22	30	
Berry and Washbourn (1900)	142	33	23	31·0 %
Thomas (1904)	1027	277	27	
Goadby (1900)	586	99	16·9	
	6020	1632	26·8	
	10570	1955	18·2	

[1] Quoted by Graham-Smith (1904).

Table (C) showing nose infection with Hofmann's bacillus amongst healthy persons.

	No. of persons examined	No. infected with Hofmann's bacillus	Percentage infected	
Adult non-contacts.				
Massachusetts Committee (1902)				
Ontario	50	7	14·0	
Boston	892	331	37·1	
Lowell	250	4	1·6	
Springfield	185	15	8·1	
Neumann (1902)	206	200	97·0	30·9 %
Hasslauer (1902)	82	23	28·0	
Stein (1900)	86	7	8·1	
Thomson and Hewlett (1895)	103	0	0	
Baurowicz (1895)	40	0	0	
Children non-contacts.				
Massachusetts Committee (1902)				
Washington	221	1	0·4	
Providence	927	140	15·1	
Pugh (1902)	420	234	55·7	25·6 %
Lack (1899)	100	52	52·0	
Children contacts.				
Massachusetts Committee (1902)				
Minnesota	1154	378	32·7	
Brookline	129	15	11·6	27·6 %
Minnesota Board of Health (1900)	393	70	17·8	
	5238	1477	28·2	

Table B relates only to the distribution of the bacillus in the throats of persons not suffering from clinical diphtheria. As far as possible the observations on adults have been separated from those on children, and those on contacts from those on non-contacts. In both cases it has been impossible to make an entirely satisfactory division, owing to the lack of differentiation in the original tables. According to the statistics of American observers Hofmann's bacillus (Wesbrook's type, D_2) is found only in small numbers in the throats of healthy non-contacts, whether children or adults. Children, however, who have been more or less exposed to diphtheria are more frequently infected. According to the investigations of European observers, Hofmann's bacillus is more commonly present in all classes. They are less numerous in both classes of adults than in children, though in each class more numerous amongst contacts than non-contacts.

Certain investigations on the occurrence of Hofmann's bacillus in the nose are quoted in Table C, mostly by American observers. They indicate that Hofmann's bacillus is more commonly present in the nose than in the throat, and that there is no difference in the degree of infection in contacts and non-contacts, adults and children.

A more careful examination of the former table shows that there is more uniformity amongst the European than the American statistics, but even amongst the former considerable differences are to be found. These differences are probably to some extent due to the different methods employed, and partly to different classes to which the persons belonged. As extreme instances, may be quoted the investigations of Neumann, and Thomson and Hewlett. The former found 98% of the 111 normal noses he examined to be infected, while the latter never found the Hofmann's bacillus in 27 cultures from the vestibulum naris, nor in 76 from the interior of the nose, 101 cultures in all. Very divergent results are also to be found in the American statistics. The collaborators in the "Report on diphtheria bacilli in well persons" by a Committee of the Massachusetts Association of Boards of Health examined the throats of 64 school children and 121 male prisoners at Springfield and found bacilli belonging to type D_2 in 13, to type E_2 in 10, and G_2 in 1, or 24 persons out of 185—(12·9%). At Providence, on the other hand, from the throats of 541 school children, 376 tramps and 10 small-pox patients only 6 bacilli belonging to the type D_2 were found (·6%), and none belonging to types E_2 or G_2—(·6%).

In consequence of the difficulty in interpreting these varying results the writer has added a table (D) showing the results of his findings in

the throats of a large number of persons at Cambridge and Colchester. These observations have the advantage of having been made by the same observer under the same conditions. All cultures have been made on a modification of Lorrain Smith's serum, and all have been examined after 12—18 hours' growth and again after a further period of 24 hours' growth. In all cases, wherever possible, single colonies or groups of similar colonies, and not smears, have been microscopically examined. The second examination has frequently resulted in the finding of isolated colonies of the Hofmann's bacillus, which had been missed on the first occasion. The fact that several investigators have confined their examinations to smears made from 18 hour old cultures probably accounts for many low figures, since in crowded cultures containing only one or two very small colonies of the Hofmann's bacillus these are very easily overlooked. Further, in this set of observations pure cultures have been made from a very large number of the colonies showing doubtful organisms and their reaction in glucose broth tested, and the effects of subcutaneous injections into guinea-pigs have been examined in many instances.

As the result of these investigations the writer has come to the conclusion that Hofmann's bacillus is found most commonly in the throats of the children of the poorer classes, especially the scholars in the public schools, the percentage infected being about the same in contacts and non-contacts (51 % and 56 %). The children attending better class schools are less commonly found to harbour this bacillus in their throats (8 % and 15 %). In adults the infection is less than in children, but it is greater amongst the poor (20 %) than amongst the well-to-do (9 %). The Hofmann's bacillus, like the diphtheria bacillus, is probably transferred from mouth to mouth by pencils, sweets, etc., which pass from one child to another, and the habit which children have of placing their fingers and articles, such as pencils, in their mouths accounts for the high infection amongst them with this organism. The absence of such habits amongst older persons may account for the smaller percentage of infection amongst them. In fact a high percentage of infection with Hofmann's bacillus is usually associated with a lack of cleanliness.

Some of the numbers on which these statements are based are very small, for example, only 97 children attending better class schools, and only 66 adults of the more well-to-do class having been examined. The difference in the numbers infected by Hofmann's bacillus in these various classes is, however, sufficiently marked to justify the opinion that more extended investigations along these

TABLE D.

	No. of persons examined	No. in whom Hofmann's bacillus found	Percentage infected with Hofmann's bacillus		Percentage infected with the diphtheria bacillus: Virulent	Not tested	Non-virulent	Remarks.
Adult non-contacts.								
Members of the University	48	2	4·1	9·0	0	—	0	
Workers in the Pathological Laboratory	18	4	22·2		0	—	0	
Patients in Hospital	98	12	12·2	20·6	0	—	1	Mostly belonging to same class as scholars of elementary schools.
Other persons	198	49	24·2		0	—	0	
Adult contacts.								
Colchester	79	18	22·7	19·0	—	29·1	—	
Post-office	52	8	15·3		1·8	—	1·8	
Printing Office	19	2	10·5		0	—	0	
Dress makers	29	7	24·1		3	—	0	
Hospital	40	6	15·0		17·5	—	2·2	
Sanatorium	49	10	20·4		15·5	—	0	
Children non-contacts.								
Cambridge School A	16	10	62·5	55·9	0	—	0	Scholars of public elementary schools.
,, ,, B	21	13	61·9		0	—	0	
,, ,, C	22	10	45·8		0	—	0	
,, ,, D	29	0	0	7·7	0	—	0	Better class schools.
Colchester School XI	10	3	30		0	—	0	
Children contacts.								
Colchester School I	6	4	66·6	51·4	0	—	0	Scholars in the public elementary schools.
,, ,, II	30	20	66·6		0	—	0	
,, ,, III	50	32	64·0		—	16·0	—	
,, ,, IV	49	31	63·3		—	16·0	—	
,, ,, V	149	94	63		—	4·0	—	
,, ,, VI	59	37	62·7		—	8·4	—	
,, ,, VII	16	10	62·5		—	6·2	—	
,, ,, VIII	15	9	60·0		—	33·3	—	
Sturton St School	120	72	60·0		4·1	—	0·8	
Ross ,, ,,	46	28	60·0		2·1	—	0	
Abbey ,, ,,	32	19	59·3		0	—	3·0	
Colchester School IX	37	21	57·0		—	13·5	—	
New St School	42	22	52·3		0	—	2·3	
Catherine St School								
Class 1	47	25 (53·1)	51·1		0	—	0	
,, 2	102	50 (49·0)			1·0	—	0	
,, 3	54	28 (51·8)			14·0	—	1·5	
,, 4	59	27 (45·7)			3·1	—	3·1	
,, 5	41	26 (63·4)			0	—	0	
Park St School	20	10	50·0		0	—	0	
St Matthew's School								
Infants (Spring)	288	105 (36·4)	37·4		5·5	—	1·2	
,, (Autumn)	49	24 (48·9)			14·0	—	0	
Girls	122	43 (35·2)			6·1	—	0·7	
Colchester School XI	9	3	38·3	15·3	—	1·0	—	Better class schools.
Cambridge ,, E	49	6	12·2		—	2·0	—	
	2219	900	40·5					

lines would confirm the value of this classification. Amongst members of the University the infection with Hofmann's bacillus is low (4·1 %). Amongst school children of the same social class the infection in non-contacts was 7·7 % and in contacts 15·3 %. Adult non-contacts, belonging to the class from which the scholars of the public elementary schools are drawn, showed infection in 20·6 % and contacts in 19 %. Amongst school children of this class, who had not been exposed to diphtheria, 55·9 % were infected, whilst amongst those who had been more or less exposed 51·4 % were infected. Many of the scholars included under the head of contacts if exposed at all had only been so to a very slight extent, as may be seen from the absence of diphtheria bacilli amongst them; and only those are called non-contacts who were examined at a time when no diphtheria was present in the town. The uniformity of the results is therefore the more striking. This point is made especially clear in the case of the Catherine Street School, where the percentage of infection with Hofmann's bacillus in all five classes is practically identical, whereas in only three were diphtheria bacilli, virulent or non-virulent, found.

In the throats of 40 % of all persons examined Hofmann's bacilli were found.

Cobbett (IV. 1901, p. 243) had previously made similar observations, remarking that Hofmann's bacillus "was certainly not more frequently found among those who had come into contact with diphtheria than among those who had not. It was less frequently found among the scholars of the 'higher grade' school, where there was much diphtheria, than among the scholars of the ordinary schools, where there was little or none. Among the small number of children of the upper classes examined it was conspicuous by its rarity."

Roux and Yersin (1890) also remarked that these bacilli were less frequently encountered in the better class children.

Cobbett has recently made a very careful examination of the diphtheroid organisms found in cultures from the throats of 90 boys in a reformatory school in which no diphtheria had occurred for some years. Hofmann's bacillus was isolated from the throats of 39 (43·3 %) and was seen in crowded cultures from the throats of some others. All the isolated organs were totally non-pathogenic for guinea-pigs, gave typical cultures on the ordinary media and produced no acid in glucose, galactose, laevulose, maltose, dextrine, lactose, glycerine, saccharose or mannite. This is the most thorough investigation which has yet been made on this subject.

The presence of Hofmann's bacillus has been recorded in many other situations besides the throat and the nose, namely, the eye, the ear, the skin, and the female genitals. This organism, or one very closely resembling it, has also been isolated from pus, the air, and from animal lesions.

The Seasonal Prevalence of Hofmann's Bacillus.

Boycott (1905) has attempted to show that Hofmann's bacillus is more prevalent at certain periods of the year than at others. The materials used were the results of 15,000 examinations made during six years (1899—1904) at the Lister Institute. "The diagnosis is based upon the microscopical appearances found in young (12—20 hours) cultures on serum. The films are made from smears taken over the whole surface of the culture and no attempt is made to pick out individual colonies."

The author states that he has "eliminated from the records those cases in which swabs were taken from persons who were merely 'contacts' of diphtheria infections. The remainder comprise those who were suffering either from true diphtheria or some affection of the throat, bearing a likeness to diphtheria sufficiently close to render bacteriological examination desirable." He concludes that there is a clear difference in the seasonal variations of positive examinations for the two organisms, diphtheria prevailing during September, October and November, while Hofmann's is more frequent from May to August. He discusses the possible errors due to no further search for Hofmann's bacillus being made after the diphtheria bacillus has once been found, and of pure cultures of diphtheria only being obtained when the swabs have been accurately taken from the membrane, and also recognises the fact that Hofmann's bacillus in the latter case may often be present in other areas of the mouth, nose, or pharynx, or, even if present on the swab, be overgrown by the diphtheria bacillus, but thinks that they do not materially affect his results. In spite of these assertions further proof is necessary before these conclusions can be finally accepted, since each of the sources of error mentioned is capable of exerting a considerable influence; and without doubt a more careful method of examination, especially after further growth, would reveal a greater percentage of persons infected with the Hofmann's bacillus. Moreover, if Hofmann's bacillus is a common inhabitant of the healthy mouth and nose, seasonal variations ought to be sought for amongst healthy persons, as well as amongst the class of persons here mentioned, before any definite conclusions can be made.

Summary of Chapter V.

The typical form of Hofmann's bacillus is easily differentiated in young cultures from the diphtheria bacillus by its morphological characters and by its lack of polar bodies when stained by Neisser's method. Even the atypical segmented forms can be readily recognised by a practised observer. In cultures it differs from the diphtheria bacillus mainly by its more luxuriant growth on the common media, and its inability to produce an acid reaction in glucose broth. It is also entirely non-pathogenic to laboratory animals in ordinary doses. The organism is a fairly common inhabitant of the healthy throat and nose, and is more commonly found in children than in adults. Many observers consider that it is more frequently present in the throats and noses of contacts than those of non-contacts, but the writer's experience and that of many other observers is entirely against this view[1].

Note.—Sholley (15. VI. 07) reports that 266 (26·6 %) out of 1000 cultures, obtained from apparently non-exposed children attending the New York hospitals, contained Hofmann's bacilli.

[1] Many of the disputed points are discussed in the next chapter.

CHAPTER VI.

THE RELATION OF THE DIPHTHERIA BACILLUS TO HOFMANN'S BACILLUS.

Experimental attempts to convert the diphtheria bacillus into Hofmann's bacillus. Experimental attempts to convert the Hofmann's bacillus into the diphtheria bacillus. Toxoid production by Hofmann's bacillus. Action of the bacilli on various sugars. Agglutination. Haemolysis. Experimental attempts to decrease the virulence of diphtheria bacilli. Experimental attempts to increase the virulence of diphtheria bacilli. Change of virulence by transference from one individual to another. Observations on the change of morphological type by various means. Hofmann's bacilli apparently virulent to man. The virulence towards guinea-pigs of Hofmann's bacilli derived from various sources. Experimental attempts to make Hofmann's bacillus virulent. Change of morphological type in Hofmann's bacillus. Opinions of recent investigators. Summary.

In the last two chapters the principal features of the diphtheria bacillus and Hofmann's pseudo-diphtheria bacillus have been dealt with. In this chapter the various views and arguments as to the relationship existing between them will be considered.

Some observers consider Hofmann's bacillus to be a common innocuous saprophyte, which is in no way related to the diphtheria bacillus. Others appear to think that more than one species of bacillus has the morphological appearance of Hofmann's bacillus and that the diphtheria bacillus is occasionally encountered in this form (see p. 130). Others regard Hofmann's bacillus as a slightly altered and attenuated diphtheria bacillus capable under some conditions of becoming converted into the genuine virulent diphtheria bacillus and giving rise to diphtheria.

Hewlett (1899, p. 203), for example, believing "that Hofmann's pseudo-diphtheria bacillus is a modified diphtheria bacillus," goes so far as to say that a positive diagnosis of diphtheria should be given whenever it is found. The views of the latter school are supported by arguments based on experiments, observations, and statistics.

Some observers have attempted to change the one organism into the other, some have attempted to demonstrate that similar products are produced by the two in culture, and others to show that they react in a similar manner towards agglutinating sera. The opinion is very widely held that diphtheria bacilli recently isolated show great differences in their virulence towards guinea-pigs, and numerous experimenters have sought to increase or decrease the virulence by artificial means. Attempts have been made to prove that one cause of decreased virulence is prolonged stay in the throat. From the results of such experiments it has been argued that the virulent diphtheria bacillus is capable of becoming naturally attenuated and finally converted into the Hofmann's bacillus, and that the Hofmann's bacillus may by a reverse process become changed into a diphtheria bacillus. Arguments in favour of these changes have also been based on statistics apparently showing that Hofmann's bacillus is more commonly found in the throats and noses of those who have recently suffered from diphtheria and those who have been in contact with the disease than in the throats or noses of the normal population.

In this chapter these experiments and deductions are examined at length in the following order.

A. Observations directly bearing on the Relationship of the Diphtheria Bacillus to the Hofmann's Bacillus.

(1) Experimental attempts to convert the diphtheria bacillus into the Hofmann's bacillus.

(2) Experimental attempts to convert the Hofmann's bacillus into the diphtheria bacillus.

(3) Observations on the production of similar substances (toxoids) in culture by the two organisms.

(4) Observations on the action of the two organisms on media containing various sugars and carbo-hydrates.

(5) Observations on the action of agglutinating and bacteriolytic sera on the two organisms: (*a*) sera of diphtheria patients, (*b*) antitoxic sera, (*c*) sera of animals immunised with cultures, (*d*) bacteriolytic sera, (*e*) haemolysis.

Summary.

B. Observations indirectly bearing on the Relationship between Diphtheria and Hofmann's Bacilli.

(1) Experiments on the virulence of diphtheria bacilli (*a*) found in the mouths of healthy persons (contacts), and (*b*) on those observed to persist for long periods in the throats of infected persons.

(2) Experimental attempts to decrease the virulence of diphtheria bacilli (*a*) by prolonged cultivation, (*b*) by heating, (*c*) by drying. (*d*) Spontaneous attenuation.

(3) Experimental attempts to increase the virulence of diphtheria bacilli (*a*) by growth with other organisms, (*b*) by passage through animals, and (*c*) by other means.

(4) Observations on changes in virulence in diphtheria bacilli by transference from one individual to another.

(5) Observations on the virulence of diphtheria bacilli causing lesions in different parts of the same individual.

(6) Observations on the changes in morphological type in diphtheria bacilli (*a*) during prolonged persistence in the throat, (*b*) by transference from one individual to another, (*c*) by growth on artificial media, (*d*) by passage through animals.

(7) Observations on the pathogenicity of Hofmann's bacillus. (*a*) Observations on Hofmann's bacilli apparently pathogenic to man. (*b*) The virulence towards guinea-pigs of Hofmann's bacilli recently isolated from (1) healthy persons, (2) persons suffering from diphtheria, (3) convalescents from diphtheria, (4) healthy persons infected with diphtheria bacilli, (5) healthy members of infected families not themselves infected with diphtheria bacilli, (6) persons suffering from throat lesions more or less resembling diphtheria.

(8) Attempts to make Hofmann's bacilli virulent for guinea-pigs (*a*) by passage through animals, (*b*) by growth with other organisms.

(9) Observations on the changes in morphological type in Hofmann's bacilli (*a*) after passage through animals, and (*b*) in culture.

(10) Evidence bearing on the relationship of Hofmann's and diphtheria bacilli derived from preventive measures.

Opinions of recent investigators.

Summary.

A. Observations directly bearing on the Relationship of the Diphtheria Bacillus to the Hofmann's Bacillus.

(1) *Experimental Attempts to convert the Diphtheria Bacillus into the Hofmann's Bacillus.*

Hewlett and Knight (1897, p. 22) attempted to convert diphtheria bacilli into Hofmann's bacilli by heating cultures. They selected a large, segmented, polar staining, acid-forming, virulent diphtheria bacillus, of which a broth culture in a dose of ·25 c.c. was capable of killing a guinea-pig in 24 hours.

Four broth cultures were prepared, and after 48 hours growth' were kept for 4, 6½, 17 and 24 hours respectively at 45° C. The last culture was killed by the heating, but in the first two the result was inappreciable. "The growth in the first subcultures obtained from the broth heated for 17 hours was very poor, showing that most of the organisms had been killed; and the nature of those that remained alive had been completely changed. In later subcultures the bacilli were short, and had lost their polar staining and segmentation." These characters were retained for nine generations (i.e. until the paper was written). Neutral litmus agar was turned blue, and anaerobic cultures in hydrogen gave no trace of growth. Doses of broth cultures up to 5 c.c. proved innocuous to guinea-pigs. The authors conclude their description with the following statement. "We have attempted to obtain the same transformation with other virulent diphtheria bacilli, but not so successfully, although in two instances, after heating, pseudo-forms were obtained; the change from Klebs-Loeffler to pseudo was not nearly so complete. The amount of heating required seems to be a very delicate factor: too little leaves the bacilli comparatively unaltered, a little more kills them completely."

On the other hand, Williams (1902) kept two cultures for several months at 40° C. transplanting them every week, and kept six cultures for several months at 43—45° C., alternating every week to a temperature of 35° C. When growing at 40—43° C. the bacilli were found to be smaller, but returned to their original condition when grown at 35° C.

Park (1900) states that the diphtheria bacillus "may grow at a temperature as high as 41° C. and retain its virulence for months, and Roux and Yersin (1890) found that no morphological alteration of a strain could be affected by growth at 39·5° C.

(2) *Experimental Attempts to convert the Hofmann's Bacillus into the Diphtheria Bacillus.*

Hewlett and Knight (1897) in their experiments made use of typical Hofmann's bacilli, which turned neutral litmus agar blue and produced no effect on guinea-pigs. One experiment is described at great length. The bacillus was obtained from a nurse, who had been nursing a case of diphtheria, and was isolated by passing through agar plates. It was cultivated on serum at 37° C. for 24—48 hours, through 18 generations. During this time its morphology does not appear to have remained constant, for sometimes it appeared to be a typical Hofmann's, at others a short diphtheria, bacillus, and occasionally a mixture of the two types was seen. The 18th generation was a typical Hofmann, producing an alkaline reaction in sugar media, and 5 c.c. of broth culture had no effect on a guinea-pig.

The 19th and 20th generations were each incubated on serum for seven days at 37° C., and from the last a broth culture was made and incubated for six days. Of this 5 c.c. inoculated into a 250 grm. guinea-pig (No. I) caused death in 36 hours with marked oedema. The bacilli were recovered from this animal and broth cultures made from them and incubated for 48 hours killed a 316 grm. guinea-pig (No. II) in 40 hours with marked oedema. The bacilli recovered from the latter killed large guinea-pigs (Nos. III and IV) of 536 and 478 grms. in 36 to 40 hours in doses of 4 c.c. and 3 c.c. The bacilli recovered from these four guinea-pigs were typical diphtheria bacilli in morphology, turned litmus media a bright red, and grew anaerobically in hydrogen. They were capable of killing half-grown guinea-pigs in small doses varying from between 1 and ·25 c.c. The authors discuss, but reject, the possibility of having started with a mixed culture, and state that "in other cases, which showed only typical pseudo-forms, both morphologically, culturally, and in non-virulence, similar treatment, viz. by incubating on serum for a week for a variable number of generations, has resulted in broth cultures a week old, inoculated in 5 c.c. doses, killing the guinea-pigs in 7 to 10 days, or a little longer. This result is so constant, and has been repeated so many times, that we are convinced that it is not merely accidental, especially as guinea-pigs inoculated with similar doses from unincubated cultures usually remain perfectly well." A consideration of the various points raised in their paper leads them "to the conclusion that the so-called pseudo-diphtheria bacillus is frequently a modified and non-virulent diphtheria bacillus."

Trumpp (1896) states that he converted a non-virulent bacillus, which he calls a pseudo-diphtheria bacillus, into one capable of killing guinea-pigs with all the symptoms of true diphtheria by successive passages of cultures mixed with non-fatal doses of diphtheria toxin through guinea-pigs. This organism originally produced acid and was therefore probably a non-virulent diphtheria bacillus.

Levin (1901) said that he converted by cultivation a non-virulent pseudo-diphtheria bacillus into a true virulent diphtheria bacillus, which showed polar bodies. The toxin produced by this organism could be neutralised by antitoxin.

In 1898 Richmond and Salter briefly stated that by repeated passages through certain birds they had been able to convert Hofmann's into diphtheria bacilli, but gave no details. These were supplied by Salter (1899) in the following year.

Nineteen strains of pseudo-diphtheria (Hofmann's) bacilli were examined. All were found to be capable of killing small birds of the finch and bunting families, of which the goldfinch, chaffinch, siskin and sparrow were the least resistant. Sixteen species of birds were experimented on, with the following results:

Goldfinch (*C. elegans*). All 19 pseudo-diphtheria bacilli pathogenic.
Chaffinch (*F. coelebs*). ,, ,, ,, ,,
Canary (*S. canarius*). 18 out of 19 pathogenic.
Siskin (*C. spinus*). 3 pseudo-diphtheria bacilli tested, all pathogenic.
Hedge sparrow (*A. modularis*). 8 pseudo-diphtheria bacilli tested, 6 pathogenic.
Robin (*E. rubecula*). 8 pseudo-diphtheria bacilli tested, 6 pathogenic.
House sparrow (*P. domesticus*). 14 pseudo-diphtheria bacilli tested, 8 pathogenic.
Greenfinch (*C. chloris*). 13 pseudo-diphtheria bacilli tested, 2 pathogenic.
Yellow Hammer (*E. citrinella*). 13 pseudo-diphtheria bacilli tested, 1 pathogenic.
Brambling Finch (*F. montifringilla*). 3 pseudo-diphtheria bacilli tested, 1 pathogenic.
Linnet (*L. cannabina*). 11 pseudo-diphtheria bacilli tested, 1 pathogenic.
Blackbird (*T. merula*). Totally insusceptible.
Thrush (*T. musicus*). ,, ,,
Hawfinch (*C. vulgaris*). ,, ,,
Starling (*S. vulgaris*). ,, ,,
Pigeon (*C. livia*). ,, ,,

It was found that as a result of two passages through a chaffinch any given stock was rendered capable of causing every linnet inoculated to succumb, whilst after two more passages through the latter bird, the thrush and the blackbird were no longer proof against the organism. Similarly, three passages through the goldfinch elevated the virulence of one strain to such an extent that it became capable of killing the blackbird in 48 hours. Finally, it was ascertained that repeated passages

through almost any susceptible bird rendered the pseudo-diphtheria bacillus pathogenic to the guinea-pig.

Details of one experiment, in which a non-virulent Hofmann's bacillus was converted into a diphtheria bacillus virulent for guinea-pigs, are given as follows. The organism was: (1) Short and plump, uniform in staining, and without polar granules. (2) Its colonies on serum were pearly white. (3) In neutral broth it produced an alkaline reaction. (4) 10 c.c. of a 48 hour broth culture, in which were emulsified two whole serum cultures, failed to kill a 230 grm. guinea-pig. (5) A filtered 7-day alkaline broth culture in a dose of 20 c.c. was innocuous to a guinea-pig.

Of this organism half a serum culture, suspended in 1 c.c. of a 48 hour broth culture, was inoculated into the left pectoral muscle of a goldfinch. On the second day the bird became quiet and died in 96 hours. Cultures were made from the site of inoculation three hours after death. There was ecchymosis and pallor of the muscle, together with definite turgidity and swelling. Bacilli slightly longer than those inoculated were found in pure culture. Nothing else abnormal was discovered in the body. This culture he labelled A. 1 c.c. of a broth subculture of A was inoculated into a second goldfinch. It died in 48 hours. The organisms found at the autopsy had become longer (culture B). A loopful of B suspended in 1 c.c. of broth was inoculated into a third goldfinch. Death occurred on the third day. At the autopsy the whole of the pectoral muscle on the side of the inoculation was yellowish white and the fibres were very friable and obviously degenerated. The culture obtained was called C. A small loopful of C was injected into a fourth goldfinch. Death took place within 48 hours. The bacilli recovered (D) assumed the typical short diphtheria form with polar bodies.

5 c.c. of a 48 hour culture of D, together with one serum culture, were injected into a guinea-pig of 250 grms. The animal suffered a large local reaction, followed by necrosis, and was ill for several weeks, but ultimately recovered. A tiny loopful of D was injected into a fifth goldfinch. It died in 36 hours. From the local lesion perfect specimens of the diphtheria bacillus were obtained. 5 c.c. of a broth culture, together with a serum culture, killed a 260 grm. guinea-pig on the fourth day with the typical lesions of experimental diphtheria. The pathological effects could be neutralised by antitoxin. Two tubes of alkaline broth were placed side by side, one from the original culture, the other from the exalted culture. The first became alkaline and the

second acid. This experiment was repeated with three other strains. He concludes that "There are to be met with diphtheritic organisms of every grade of virulence. The weakest, known as Hofmann's or the pseudo-diphtheria bacillus, and representing the most attenuated form of the diphtheria bacillus, is capable of killing only certain highly susceptible birds of the finch tribe. Organisms of a slightly higher degree of virulence can kill other and more resistant small birds of the bunting family. Yet others, still more active, can cause larger birds (*Merulidae*) to succumb, while the most virulent of all can kill certain of the rodents, such as the guinea-pig."

The pathogenic action of Hofmann's bacillus on small birds has not been investigated by many observers. Simonin and Benoit (10. 1. 1898) state that it is capable of killing a small exotic bird, called "Calfat," and Hewlett (1904) has attempted to enhance the virulence of Hofmann's bacilli by passing them through small birds, but no positive results were obtained. He says that when recently isolated it is virulent for the chaffinch, but by continued cultivation on agar it loses its virulence even for this bird. On the other hand, Williams (1902, p. 107) investigated the subject and found that even in large doses four strains of Hofmann's bacillus and two of the non-virulent diphtheria bacillus were perfectly innocuous to goldfinches.

(3) *Observations on the Production of Similar Substances (Toxoids) in culture by the two Organisms.*

Salter (1899) found that the filtrate of 7—10 days old broth cultures of Hofmann's bacillus although without effect on guinea-pigs in doses of 20—30 c.c., yet in doses of 1—1·5 c.c. was capable of killing chaffinches and goldfinches[1]. Control birds treated with corresponding quantities of sterile broth remained unaffected. He therefore considered that we might legitimately speak of a toxin of the Hofmann's bacillus. He further found that the filtrate, although harmless to guinea-pigs, had in a marked degree the power of neutralising or fixing antitoxic sera. The experiments on which this opinion was based are given below in detail.

A series of seven guinea-pigs were injected with varying quantities of a diphtheria toxin and 1 unit of antitoxin, with the following results :—

[1] Salter (1899) states that if a subcutaneous injection of a Hofmann's bacillus be given to a bird a large local gelatinous effusion occurs. Suprarenal reddening is never seen in birds even when inoculated with diphtheria bacilli.

	Weight	Dose	Result
Guinea-pig 1	250 grms.	·35 c.c. of toxin and 1 anti-unit	Died 3 days
,, 2	255 ,,	·34 ,, ,, ,, ,,	,, 3 ,,
,, 3	252 ,,	·33 ,, ,, ,, ,,	,, 4 ,,
,, 4	255 ,,	·32 ,, ,, ,, ,,	Recovered
,, 5	258 ,,	·31 ,, ,, ,, ,,	,,
,, 6	250 ,,	·30 ,, ,, ,, ,,	,,
,, 7	245 ,,	·29 ,, ,, ,, ,,	,,

A second series were treated with similar solutions, containing in addition, however, varying amounts of a filtered broth culture of Hofmann's pseudo-diphtheria bacillus.

	Weight	Dose	Result
G.-pig 8	260 grms.	·32 c.c. of toxin and 1 anti-unit and 1 c.c. of pseudo-diphtheria filtrate	Died 3 days
,, 9	250 ,,	·32 ,, ,, ,, ,, ·5 ,, ,, ,, ,,	,, 3 ,,
,, 10	245 ,,	·32 ,, ,, ,, ,, ·25 ,, ,, ,, ,,	,, 4 ,,
,, 11	255 ,,	·31 ,, ,, ,, ,, ·5 ,, ,, ,, ,,	,, 4 ,,
,, 12	248 ,,	·30 ,, ,, ,, ,, ·5 ,, ,, ,, ,,	,, 5 ,,
,, 13	252 ,,	·29 ,, ,, ,, ,, ·25 ,, ,, ,, ,,	,, 8 ,,
,, 14	250 ,,	·29 ,, ,, ,, ,, ·5 ,, ,, ,, ,,	Recovered
,, 15	255 ,,	·28 ,, ,, ,, ,, ·5 ,, ,, ,, ,,	Died 6 days
,, 16	240 ,,	·26 ,, ,, ,, ,, ·5 ,, ,, ,, ,,	Recovered

He states that the toxin antitoxin mixture was always prepared first and allowed to stand for a few minutes, before the pseudo-diphtheria filtrate was added. The filtrate must therefore have possessed the capacity of breaking up the neutralised combination, liberating the toxin, and taking its place; that is to say, it was a Protoxoid.

Lesieur (1901) alleges that certain strains of "pseudo-diphtheria" bacilli, although non-virulent for guinea-pigs in ordinary doses, can nevertheless cause fatal paralyses similar to those due to diphtheria toxin, provided that large doses of recently isolated cultures be used. He evidently appears to consider that the paralysing substance is a very similar body, if it is not indeed identical, with the toxone in diphtheria toxin. These experiments have, however, not been confirmed by more recent observers.

Cobbett (1903, *Journ. of State Med.* p. 609) and Hewlett (1904) have been unable to find any toxoids in cultures of Hofmann's bacillus. The most thorough and convincing observations are those of Petrie (1905), who, working with 11 races of Hofmann's bacillus, mostly derived from diphtheria patients or contacts, which showed no polar granules by Neisser's method, produced no acid in glucose broth, and

were non-virulent to guinea-pigs in doses of 5 c.c., made several experiments to ascertain whether any toxoid was produced.

One race (No. 1) was grown in a large volume of alkaline broth in the usual manner for the production of diphtheria toxin, and a second culture of the same race was grown in Martin's pig-stomach bouillon at 36° C., and after 10 days' growth the cultures were filtered through Pasteur-Chamberland filters. A mixed filtrate from the other 10 races was also prepared. Tables are given in his paper showing the results of the 33 experiments similar to those made by Salter, carried out with a view to ascertaining whether toxoids were present in the filtrates. A quantity of filtrate was first added to a unit of antitoxin and the mixture then placed in the incubator for 15 minutes before the addition of the toxin. In this way any toxoid which might be present had an opportunity of combining with the antitoxin, thus preventing a corresponding amount of toxin from entering into combination. A further series of 11 experiments were made in which the dose of toxin was such that toxone effect became manifest in 3 or 4 weeks. He comes to the conclusion that "Taken as a whole these experiments may be interpreted as indicating that no toxoids were present in the filtrates of the pseudo-diphtheria (Hofmann's) bacilli examined." Taking the view that if toxoid is really present as a product of metabolism of Hofmann's bacillus the immunisation of a suitable animal with these products ought to lead to the production of an antitoxin in the animal's serum, he made the following experiments. Three horses were repeatedly injected with large quantities of the two filtrates mentioned. Before and after the experiments their blood was tested for antitoxin and none was found. Consequently the filtrates employed were not capable of producing an antitoxin to diphtheria toxin. As the result of his experiments Petrie concludes: (1) that no substances capable of neutralising diphtheria antitoxin are present in filtrates of Hofmann's pseudo-diphtheria bacilli, and (2) that the results of the immunisation of horses with large quantities of the filtrates make it apparent that they do not contain substances capable of stimulating the production of an antitoxin to diphtheria toxin.

Glücksmann (1897), Bergey (1898) and others had previously ascertained that the immunisation of animals with cultures of Hofmann's bacillus did not confer any protection against diphtheria bacilli injected subsequently, and Spronck (1896) had found that the local reaction produced in guinea-pigs by the subcutaneous injection of large doses of pseudo-diphtheria bacilli was not influenced by the injection of anti-

toxin. One or two instances are, however, quoted, in which a very slight degree of protection seems to have been produced against diphtheria bacilli by immunisation with Hofmann's bacillus.

(4) *Observations on the Action of the two Organisms on Media containing Various Sugars and Carbohydrates.*

Experiments have already been quoted (p. 159) which show that diphtheria bacilli, whether virulent or non-virulent, derived from clinical cases, convalescents or contacts, invariably produce acid in the presence of glucose, galactose and laevulose, generally with maltose, glycerine, dextrine and lactose, and occasionally with saccharose, but never with mannite, when these substances are contained in Hiss's serum medium or in broth free from muscle sugar. These experiments give no indication that the power of reacting on these substances is gradually lost during a prolonged stay in the throat[1].

Hofmann's bacillus, on the other hand, does not produce acid in the presence of any of these substances under similar conditions. If the contention that Hofmann's bacilli are merely attenuated diphtheria bacilli be true, it would seem probable that some of these organisms, when isolated from the throats of clinical cases or convalescents, would still be capable of producing acid with glucose. The experiments of many observers prove that this is not the case. During the last five years the writer has systematically isolated all the morphologically atypical forms of this organism, and large numbers of typical forms, and tested their reaction on glucose broth, but has never found a race capable of producing acid. Recently (Graham-Smith, 1906) experiments were also made with 20 races on media containing the substances mentioned above. Three of these races were derived from clinical cases of diphtheria, four from convalescents, four from contacts also infected with diphtheria bacilli, three from persons suffering from sore throat, and the rest from healthy throats and noses. None of these races showed any power of forming acid from any of these substances. These experiments, therefore, do not lend any support to the view that Hofmann's bacilli are merely attenuated diphtheria bacilli.

[1] Some writers say that the acid producing power of the diphtheria bacillus is uncertain and of no value in differentiation, but most of them seem to refer to its action on ordinary broth without the addition of glucose. Under such conditions whether acid is produced or not depends on the quantity of muscle sugar present.

In connection with these experiments a statement by Theobald Smith (1896) is of interest. He says: "An extended experience in testing the acid producing function of bacteria leads me to state that if, as has been claimed, the pseudo-diphtheria bacilli do not act on dextrose, there exists a most profound difference between them and true diphtheria bacilli, sufficient to separate them once and for all."

(5) *Observations on the Action of Agglutinating and Bacteriolytic Sera on Diphtheria and Hofmann's Bacilli.*

Numerous observations have been made with the view of ascertaining whether diphtheria bacilli can be differentiated from other diphtheria-like organisms by their reaction to the sera of patients suffering from diphtheria, antitoxic sera, or the sera of animals treated with cultures. No satisfactory results have been yet obtained with the first two sera. One of the difficulties in this method of investigation is the tendency of diphtheria bacilli to adhere in smaller or larger masses, and the impossibility of making completely homogeneous emulsions.

(*a*) *The action of the sera of diphtheria patients.*

Bruno (1898) investigated the agglutinating action of the sera of 44 diphtheria patients on young cultures of the diphtheria bacillus, both by the hanging drop and sedimentation methods. Two of these sera had no agglutinating action, 23 agglutinated in dilutions varying from between 1 : 10 and 1 : 80, 18 acted at about 1 : 100, and one at 1 : 400. He found that the serum of healthy individuals never had an agglutinating value above 1 : 30, but that after the injection of antitoxin it rose to double this value in some cases.

(*b*) *The action of antitoxic sera.*

Landsteiner (1897), Nicolle (1898), and Blandi (1903) failed to discover any agglutinating power in antitoxic serum. Nicolas (1896–98 and 1900), Fraenkel (1896), and Lesieur (1901) all found some agglutinating action, but it was inconsistent and variable and of no service in differentiating the organisms.

(*c*) *The action of the sera of animals immunised with cultures.*

Lubowski (1900) obtained an agglutinating serum by injecting a goat with non-virulent diphtheria bacilli. The bacilli were emulsified

in ·85% salt solution by vigorous shaking together with glass, and the suspension thus obtained was thoroughly mixed with 10% glycerine solution. Of this 1 c.c. was placed in a small test-tube and mixed with 1 c.c. of a suitable dilution of serum. After thorough mixing the contents were poured into a small Petri dish and examined under a low power. If the emulsion was satisfactory, it was put into an incubator and examined from time to time. The mean time of reaction was 1—2 hours. By this method 23 races of typical virulent diphtheria bacilli were examined and two races of non-virulent diphtheria bacilli. All these were agglutinated in dilutions of 1 : 80, and in some cases up to 1 : 160. Neither cocci nor Hofmann's bacilli (three races) were agglutinated. Normal serum was found in some cases to agglutinate up to 1 : 40.

Lesieur (1901) studied the action of the sera of horses, immunised by cultures of diphtheria bacilli, on 40 races of diphtheria, and 30 of pseudo-diphtheria bacilli, and found that while certain varieties of diphtheria bacilli were agglutinated others were not. The pseudo-diphtheria bacillus behaved in the same manner. He considers that the fact constitutes a new presumption in favour of the identity of certain species of pseudo-diphtheria bacilli with the true diphtheria bacilli, but as 50 (29 virulent and 21 non-virulent) of his 70 races were not agglutinated the only allowable deduction seems to be that the agglutination reaction has no value as a means of distinguishing between them.

Schwoner (1902) immunised a horse first with dead and then with living cultures of diphtheria bacilli, and obtained a highly powerful agglutinating serum. For the purpose of control he also experimented with normal horse and antitoxic sera. The latter two agglutinated between 1 : 10 and 1 : 40. He found that all the 50 races of diphtheria bacilli with which he worked were agglutinated by his serum up to 1 : 500 and some even up to 1 : 10,000. It, however, only agglutinated pseudo-diphtheria bacilli in the same strength as normal horse or antitoxic sera. Other organisms, such as *B. coli*, *B. typhosus*, and *V. cholerae* were also agglutinated at this dilution. He also immunised a goat with a pseudo-diphtheria bacillus. Its serum agglutinated that race in 1 : 10,000, but not four other strains of pseudo-diphtheria bacilli or any of his diphtheria bacilli. As the result of his observations he comes to the following conclusions: (1) That an agglutinating serum of high value can be obtained from diphtheria bacilli. (2) That a difference can be obtained between

true and pseudo-diphtheria bacilli. (3) That a pseudo-diphtheria agglutinating serum can also be obtained; and (4) That the pseudo-diphtheria bacillus is not one true race.

One of his tables shows 45 races of diphtheria bacilli agglutinated in dilutions of 1 : 1000 to 1 : 4000 in every case, and another table shows 14 races agglutinated in dilutions of 1 : 5000 and 1 : 10,000.

In 1903 Schwoner made some further experiments with 12 strains of pseudo-diphtheria bacilli. He describes 11 races of his pseudo-diphtheria bacilli, some of which are apparently Hofmann's and others non-virulent diphtheria bacilli. He divides them into two groups:—

(*a*) Culturally and morphologically Hofmann's bacilli which produce an alkaline reaction in broth, a good growth on agar, and can be easily differentiated from diphtheria bacilli.

(*b*) Morphologically like diphtheria bacilli producing a poor growth on agar and a slightly alkaline, or even acid, reaction in broth.

He made a polyvalent serum in a goat from four races of Hofmann's bacilli which agglutinated these four strains and three others in dilutions of 1 : 1000. The other races were not agglutinated. Three monovalent sera, made by injecting a goat and two rabbits, only acted well on the races from which they were made.

Gordon (1902) made agglutinating sera in guinea-pigs by the injection of killed cultures. He found that a diphtheria serum agglutinating in a dilution of 1 : 100 did not agglutinate Hofmann's bacillus, and that similarly a Hofmann agglutinating serum did not act on diphtheria bacilli. One diphtheria agglutinating serum reacted on four out of six freshly isolated virulent diphtheria bacilli and one non-virulent diphtheria bacillus, but had no action on two diphtheria-like organisms, or on Hofmann's bacilli or *B. coryzae segmentosus.*

Blandi (1903) also obtained a diphtheria agglutinatiug serum by the intraperitoneal injection of heated cultures.

Schick and Ersettig (1903) worked with 50 races of diphtheria bacilli. Of these 12 were tested for virulence and all killed within 72 hours. They obtained an agglutinating serum from a horse, which agglutinated all 50 races in dilutions of 1 : 2000, but had no effect on various other bacilli.

Martin (1903) obtained sera reacting at 1 : 200 by intravenous or intraperitoneal injections of bacilli killed by heating to 100° C. He found that the most convenient method of carrying out the agglutinating experiments was with bacilli heated in fluid to 100° C. for

one hour and emulsified with water or normal saline solution. The coarser particles were allowed to sediment and the upper layers used.

Lipstein (1903) obtained agglutinating sera from rabbits by the injection of virulent bacilli together with antitoxic serum. He found that the sera so obtained acted in high dilution only on the race used to immunise the animal, and not on other races of diphtheria bacilli. When two races were used for immunisation the resulting serum agglutinated them both in equal dilutions, but not other races. He explains these results by supposing that all races have certain receptors in common, but that in addition each race has receptors peculiar to itself.

Bacteriolytic sera.

Lambotte (1902) injected guinea-pigs first with emulsions of diphtheria bacilli killed by heating to 60° C., and later with living cultures at eight day intervals. The serum was then found to contain a specific bacteriolysin for diphtheria bacilli. The serum of guinea-pigs treated with Hofmann's bacillus contained a specific bacteriolysin for that organism. He found that the serum for one organism acted to some extent on the other, and consequently thinks that they are connected.

Haemolysis.

Lubenau (1901) observed that cultures (six out of seven) of diphtheria bacilli were capable of producing haemolysis. The maximum haemolytic power was shown in broth cultures at periods varying between 1 and 13 days.

Schwoner (1904) made more extended observations on 70 cultures. He found that the haemolytic power was best shown on rabbits' corpuscles, and as a result of his experiments concluded that:—

(1) It is chiefly strains of diphtheria bacilli from clinically severe septic cases which give haemolysis; (2) the power is at its maximum in 48 hour broth cultures; (3) the property is lost by long cultivation; (4) filtrates of cultures show no haemolysis; (5) the pseudo-diphtheria bacillus does not possess any lysin.

Summary of Section A.

In the preceding pages it has been shown that Hewlett and Knight lay claim to having on one occasion converted by means of heating

a virulent diphtheria bacillus into a Hofmann's bacillus. These authors, however, were apparently unable to repeat the process, and Williams was unable to confirm their results, and no other observers have recorded changes in cultures kept at high temperatures. Hewlett and Knight, by prolonged cultivation, and Salter, by passage through small birds, consider that they have converted Hofmann's bacilli into diphtheria bacilli. No subsequent observers, however, appear to have been able to repeat successfully the former experiments, and, although Hewlett states that he has found the Hofmann's bacillus pathogenic to chaffinches, Williams denies that it has any action on the goldfinch, the most susceptible of all birds according to Salter.

At present therefore too much importance must not be attached to these experiments unless they can be successfully repeated.

In regard to the production of culture products by the Hofmann's bacillus of a nature similar to those of the diphtheria bacillus, Salter considered that the Hofmann's bacillus formed a protoxoid. No subsequent observers have found any trace of this substance, and the exhaustive experiments of Petrie appear to demonstrate conclusively that no such substance is produced. Other observations show that no immunity against the diphtheria bacillus can be produced by means of the Hofmann's bacillus. These experiments therefore, so far as they go, point to the non-identity of the two organisms.

The observations on the power of the two organisms to produce acids from certain sugars and carbohydrates prove that in this respect they are totally distinct. While the diphtheria bacillus is capable of acting on several of them, Hofmann's bacillus is incapable of producing acid in the presence of any of them.

The observations of Lubowski, Schwoner, Gordon, Schick and Ersettig on agglutinating sera all point to a specific difference between the two bacilli, and so do the haemolytic experiments of Schwoner. Although Lesieur thought that his observations were in favour of the identity of the two organisms, his experiments are of very doubtful value. Lambotte's bacteriolytic experiments seem to show that the two organisms are connected, but not specifically identical.

The observations relating to the formation of toxoids, acid production, and the action of agglutinating sera, with few exceptions, tend to show that diphtheria and Hofmann's bacilli belong to different species, only related to each other in being members of the same group.

B. Observations indirectly bearing on the relationship between diphtheria and Hofmann's bacilli.

(1) *Experiments on the Virulence of Diphtheria Bacilli found*

(a) *In the mouths of healthy contacts.*

It has already been shown that the great majority of observers consider that diphtheria bacilli recently isolated from clinical cases display considerable differences in their virulence towards guinea-pigs. Further, it has been constantly asserted that the diphtheria bacilli, which remain after the clinical symptoms are over, gradually lose their virulence, and may at length after a prolonged stay in the throat become completely non-virulent to laboratory animals. In fact it has been generally considered that diphtheria bacilli of all grades of virulence may be met with in the throats of convalescents and other persons. By many it has been further supposed that the representatives of the originally virulent diphtheria bacilli after becoming changed into non-virulent diphtheria bacilli undergo further changes and become Hofmann's bacilli.

In view of these statements it becomes important to consider how far the reputed change in virulence is supported by experimental evidence. Certain reasons have already been given for thinking that intermediate stages of virulence are probably of much less common occurrence than has generally been supposed, and that lack of uniformity in methods may account for many of the statements which have been made. The bacilli obtained from patients showing all the symptoms of clinical diphtheria are usually found to possess a high degree of virulence, and it is unnecessary to quote the numerous experiments which have been made. Mention must, however, be made to the fact that attenuated and non-virulent bacilli have on rare occasions been isolated from such cases.

In the great majority of cases the bacilli found in the throats of persons who have come into contact with diphtheria patients, but show no signs of the disease, are also fully virulent, though some are non-virulent. The virulent bacilli of the patients do not therefore seem to suffer a diminution in virulence by transference to immune individuals. The results of the observations of some of those workers, who have tested the virulence of the bacilli obtained from recent contacts in a considerable number of cases, are quoted below.

Park and Beebe (1894) examined 48 healthy children in 14 infected families and found diphtheria bacilli in over 50%. Of six cultures

tested all were virulent. Also five out of six infected contacts discovered in a foundling hospital showed virulent bacilli.

Aaser (1895) in an outbreak of diphtheria in a cavalry regiment examined 89 well persons. Seventeen of these harboured diphtheria bacilli, and all were fully virulent. He also examined the contacts in two scarlet fever wards in which cases of diphtheria were present. All the diphtheria bacilli which he isolated and tested were virulent.

Bolton (1896) amongst 214 persons who had been more or less exposed to diphtheria found the virulent bacillus in 41·5 %.

Müller (1897) examined the children in a general ward into which cases of diphtheria had been admitted. Twelve children with diphtheria bacilli were discovered. Six of these were virulent and six attenuated or non-virulent.

Kober (1899) found that 15 out of 128 contacts showed the presence of diphtheria bacilli, all of which were virulent.

Cobbett (1901) tested for virulence nine pure cultures obtained from 19 contacts. Of these six were fully virulent and three entirely non-virulent.

Graham-Smith (1904) during an outbreak at Cambridge isolated and tested for virulence the diphtheria bacilli found in 56 well persons who had come into more or less close contact with cases of diphtheria. Of these 38 harboured fully virulent, and 18 totally non-virulent, diphtheria bacilli[1].

Pennington (1907) tested the bacilli obtained from 18 undoubted contacts, using 24 hour serum cultures for inoculation. One strain was fully virulent, 10 more or less attenuated, and seven totally non-virulent.

Table showing the virulence of diphtheria bacilli isolated from recently infected contacts:

Observer	No. of diphtheria bacilli tested for virulence	Fully virulent	Totally non-virulent or causing a slight infiltration
Park and Beebe	12	11	1
Aaser	17	17	0
Meade Bolton	88	88	0
Müller	12	6	6
Kober	15	15	0
Cobbett	9	6	3
Graham-Smith	56	38	18
Pennington	18	1	7
	227	182 (80·1 %)	35 (15·3 %)

[1] For details of these experiments see Chapter XII.

These records clearly show that the majority of well persons harbouring diphtheria bacilli, which have been derived by contact from clinical cases, retain in their throats fully virulent organisms, and that the transference of a virulent diphtheria bacillus from a diseased to an immune person does not tend to weaken its virulence.

There is also some evidence to show that non-virulent diphtheria bacilli when transferred from one person to another do not become virulent, or give rise to the disease. In the Cambridge outbreak just mentioned the writer (Graham-Smith 1904) found in three households 5, 4, and 2 persons respectively harbouring non-virulent diphtheria bacilli without the clinical signs of diphtheria. They were only examined by accident, as two of these persons had slight sore throats. Further 13 other persons with non-virulent diphtheria bacilli remained at their homes and constantly mixed with other persons. No clinical cases of diphtheria are known to have arisen from such persons. Cobbett (1901) had a similar experience, and Park and Beebe (1895) state that typical non-virulent diphtheria bacilli are probably incapable of causing diphtheria, for the 24 cases in which they were found by them never developed any lesions, nor were they the origin of any case of diphtheria, so far as could be ascertained.

(*b*) *Observations on the virulence of diphtheria bacilli persisting for long periods in the throats of infected persons.*

In regard to the alleged general decrease in virulence by prolonged stay in the throats of infected persons certain experiments show that a decrease has probably taken place, but many other experiments very conclusively prove that the organisms may persist in the throat or the nose for very prolonged periods without showing any decrease in virulence. A number of these observations are given in the following pages.

Tobieson (1892) tested for virulence 19 strains of diphtheria bacilli found up to 31 days after the disappearance of the membrane. Of these 16 strains killed guinea-pigs between 24 and 50 hours. Two caused tumours and the animals died later. One produced early necrosis, paralysis after five weeks, and death in seven weeks. Dowson (1893) observed fully virulent diphtheria bacilli in the noses of two children during five months; and Belfanti (1894) found a boy harbouring fully virulent diphtheria bacilli seven months after the attack. Ritter (1894) observed fully virulent diphtheria bacilli in one-third of 324 diphtheria cases after the disappearance of the membrane. In four

cases they were present five weeks after the disappearance of the membrane. Gladin (1895) found virulent diphtheria bacilli present in two cases for 33 days. In another case non-virulent diphtheria bacilli were found on the 45th day. Schäfer (1895) discovered virulent bacilli in the throat of a boy 7½ months after an attack of tonsillitis. Martha (1895) found in the throat of a patient nine weeks after the attack bacilli which caused oedema but did not kill, and came to the conclusion that their virulence had been decreased by treatment.

Sevestre and Méry (1895) observed two cases in which virulent diphtheria bacilli were present 49 and 38 days after the disappearance of the membrane, and Symes (1895) in a case of rhinorrhoea, following scarlet fever, found virulent diphtheria bacilli for seven weeks. Washbourn and Hopwood (1895) found virulent diphtheria bacilli in the throat of a nurse for six weeks. Williams (1896) observed a case in which diphtheria bacilli persisted for 157 days. Up to the last they were fully virulent.

Hewlett (1896) isolated from a boy, 22 weeks after an attack of diphtheria, bacilli which in doses of ·5 c.c. and ·25 c.c. killed 500 grm. guinea-pigs in 36 hours, and Müller (1897) observed fully virulent diphtheria bacilli in the mouth of a contact for 2½ months. Stone (1897) tested 18 strains of diphtheria bacilli isolated from cultures obtained from persons 3 weeks after the disappearance of all clinical symptoms. The bacilli were grown for 48–120 hours in sugar free broth, of which 1 c.c. was injected into 800 grm. guinea-pigs. Fourteen strains were virulent killing between 24 hours and 7 days, the majority within 3 days.

Smith and Walker (1897) made careful experiments on the toxin producing power of 11 races of diphtheria bacilli obtained from the throat at periods varying between 15 and 62 days from the beginning of the disease. The results of these observations are given in the following table:

Interval in days from the earliest symptoms	Minimal fatal dose of toxin
17	·12 c.c.
15	·08
27	·08
42	·08
57	·08
62	·08
16	·07
22	·07
22	·06
26	·05
62	·05

They finally say "we observe that, so far as toxin production is concerned, the length of time the bacilli have sojourned in the throat has no tendency to reduce it below the average."

Russel (1899) in two patients found virulent diphtheria bacilli 59 days and 4½ months after the disappearance of the membrane.

Cobbett (1901) went further than the majority of the observers who have been quoted, and isolated and examined for virulence the bacilli from a number of persons on several occasions until their disappearance. Bacilli were isolated and found virulent in one person on the 2nd, 23rd, and 30th days, and in another on the 1st, 30th, 36th, and 66th days. In two contacts the bacilli were found to be virulent in one on the 15th, 36th, and 58th days and in the other on the 1st, 18th, 28th, 60th, 69th, and 82nd days. The bacilli from three other contacts who harboured non-virulent bacilli were also tested on more than one occasion, and were always found to be non-virulent. The bacilli from one of these were examined on the 1st and 31st days, from another on the 24th and 34th days, and from the third on the 1st, 21st, 24th, 34th, 63rd, 65th, 67th, 84th, 88th, and 93rd days.

Prip (1901) examined the bacilli found after the disappearance of the membrane in eight persons up to their final disappearance. In these cases full virulence was maintained up to the time of the disappearance of the bacilli, in one case up to 335 days. In two other cases, however, the bacilli showed a decrease in virulence. In one case the bacillus isolated on the 142nd day was virulent, but non-virulent on the 530th day, and in the second case although virulent bacilli were obtained on the 13th day, those isolated on the 293rd day were non-virulent.

Williams (1902) found that the bacilli from convalescent cases, isolated two and sometimes three times to test their virulence, retained their pathogenic properties until their final disappearance. Pure cultures were always isolated from the first serum cultures as well from the tubes containing the last diphtheria bacilli, each day's tubes containing 24 hours' growth being kept in the ice box until the next cultures were examined.

Pugh (1902, p. 308) found virulent diphtheria bacilli in the throat of a nurse for nine weeks after she had been nursing a case of diphtheria. This nurse had never suffered any impairment of health, and continued to harbour the virulent bacilli in spite of a month's vigorous local treatment, followed by some weeks at the sea-side.

Wesbrook (1905) found virulent diphtheria bacilli present in three

cases on the last occasion on which they were isolated and tested, namely on the 46th, 72nd, and 80th days.

Pennington (1907) tested the bacilli obtained from 25 convalescents. "In several instances the organisms were isolated at different times during convalescence and their virulence tested for a diminution in activity. In only one case was such a loss of virulence noted."

In the following table the longest periods of persistence of *fully virulent diphtheria bacilli* recorded by various observers is given:

Prip	335 days	Pugh	63 days
Schäfer	230 ,,	Smith and Walker	62 ,,
Belfanti	215 ,,	Symes	49 ,,
Williams	157 ,,	Sevestre and Méry	49 ,,
Hewlett	154 ,,	Washbourn and Hopwood	42 ,,
Dowson	150 ,,	Ritter	35 ,,
Russel	137 ,,	Gladin	33 ,,
Cobbett	82 ,,	Tobieson	31 ,,
Wesbrook	80 ,,	Stone	21 ,,
Müller	75 ,,		

The observations which have just been quoted conclusively prove that diphtheria bacilli are capable of retaining their virulence during very prolonged persistence in the throats of infected persons. In a few instances they appeared to have lost their virulence to some extent, but in the great majority they have retained their full virulence up to the time of their final disappearance.

(2) *Experimental Attempts to decrease the Virulence of Diphtheria Bacilli.*

With the majority of pathogenic bacteria it is possible by various means to increase or decrease their pathogenic powers, and working on these lines many observers have sought to influence the virulence of the diphtheria bacillus. Although these experiments when successful only prove that alterations of virulence can be brought about by artificial means, many seem to have regarded the results as lending support to the view that the virulent diphtheria bacillus is related through the non-virulent diphtheria bacillus to Hofmann's bacillus.

(*a*) *By prolonged cultivation on artificial media.*

Many observers have noticed that even after very prolonged cultivation on artificial media the diphtheria bacillus does not tend to show

any appreciable loss in pathogenic properties, though some have noticed a diminution in certain instances. Williams (1902, p. 105), for example, grew one culture for six years on artificial media and found that there was an apparent slight loss of virulence. Since "its virulence on broth averaged one two-hundredths cubic centimeters" this apparent loss of virulence after very prolonged cultivation is of little importance. Three other strains of bacilli grown on artificial media for one year all retained their original virulence. Two other cultures grown for some time on hard-boiled egg showed distinct morphological changes, the bacilli becoming exceeding short and looking like diplococci, but when transferred to other media they immediately regained all their original characteristics.

Peters (1896) found that long diphtheria bacilli did not show any decrease in virulence after prolonged cultivation, but says that the short diphtheria bacillus readily loses its virulence on culture. Bardach (1895) found that a culture lost some of its virulence after two years' artificial cultivation.

(*b*) *By heating.*

It has already been shown that Hewlett and Knight on one occasion succeeded in completely changing the morphology and virulence of a diphtheria bacillus by heating cultures, but were unsuccessful in later attempts. On the other hand Williams (1902) was unable to alter the virulence by heating, even to 45° C., and Park (1900) found that virulence was retained for months at 41° C. Roux and Yersin (1890) observed that by cultivation on broth at 39·5° C. for a month virulent diphtheria bacilli could apparently be deprived of their virulence. They found that broth cultures kept at 39·5° C. for 25 days did not kill when injected directly, but subcultures from these flasks killed quickly. Consequently no real attenuation had taken place. On repeating these experiments in a slightly different way attenuation however resulted. Broth cultures were grown at 39·5° C. until obvious development had occurred. The temperature was then raised to 40° C. In one case after 13 days of such treatment a subculture only caused oedema, and in another instance subcultures were non-virulent after 23 days. They consider that loss of virulence only occurs shortly before the death of the organisms.

(*c*) *By drying.*

Roux and Yersin (1890) observed some diminution in virulence in bacilli kept for a very long period of time in dried membranes. In one

case numerous colonies of virulent diphtheria bacilli were obtained from a membrane after a month's drying, but after five months only attenuated bacilli were found. Bacilli from another membrane kept under the same conditions showed no sensible diminution in virulence after three months.

Golowkoff (1895) thought that the virulence of bacilli kept on cloth was diminished, but Abel (1895) on the other hand found no decrease in virulence in bacilli dried on silk threads after 86 days.

(*d*) *Spontaneous decrease in virulence.*

Roux and Yersin (1890) twice observed rapid spontaneous loss of virulence in culture.

(3) *Experimental Attempts to increase the Virulence of Diphtheria Bacilli.*

The observations recorded in this section deal only with attempts to raise the virulence of living diphtheria bacilli for animals when injected in the usual manner, and not with attempts which have been made to produce an increase of the production of toxins in culture media. The latter question is dealt with later (Section V).

(*a*) *By growth with other organisms.*

Several attempts have been recorded to increase the virulence of diphtheria bacilli by cultivating them together with other organisms for more or less prolonged periods. Most of these experiments, of which one noteworthy example is quoted, have yielded negative results. Williams (1902) grew two morphologically typical, but non-virulent, strains of diphtheria bacilli together with virulent streptococci through 90 generations, transplanted every three or four days, but when separated no change in virulence or other characteristics were noted.

(*b*) *By passage through animals.*

Williams (1902) attempted to give virulence to several strains of non-virulent diphtheria bacilli by successive peritoneal inoculations, but in no instance did any increase in virulence occur. She also passed two non-virulent morphologically typical bacilli through goldfinches, but the organisms when recovered from the birds were still non-virulent for guinea-pigs.

Lubowski (1900) and Bergey (1898) could not make non-virulent diphtheria bacilli virulent by repeated passages through guinea-pigs, and Fibiger (1895) was unable to raise the virulence of non-pathogenic diphtheria bacilli by injecting them together with streptococci. Cobbett (1901, p. 497) found that a minute abscess sometimes formed at the site of inoculation after the injection of non-virulent diphtheria bacilli. "From these little abscesses the bacilli in pure culture were several times obtained and tested on guinea-pigs to see if they had gained in virulence. One of them was passed through four animals in succession. In each case the injection of 2c.c. of 48 hours broth culture produced no more effect than at first.[1]"

The writer has recently experimented with five strains of morphologically typical non-virulent diphtheria bacilli. These were injected subcutaneously into white rats, and three were recovered after 2, 2 and 5 days. Attempts to recover the other two strains after 2 and 3 days failed, and in no case could the bacilli be cultivated from the blood and organs. The recovered bacilli were found to be totally non-virulent to guinea-pigs in doses of 2 c.c. of 48 hour broth cultures.

Park (1900) says that "the passage of diphtheria bacilli through the bodies of susceptible animals does not increase their virulence to any considerable extent, this being probably due to the fact that the bacilli multiply but little in the tissues."

Although the attempt to give virulence to entirely non-pathogenic diphtheria bacilli has usually been unsuccessful, many investigators claim to have considerably raised the virulence of bacilli which had either been only feebly virulent when first isolated, or had subsequently become so.

Martin (1898) passed bacilli through the peritoneum of the rabbit in collodion sacs, and was enabled by this treatment to augment the toxigenic power, though the passage did not increase the virulence.

Roux and Yersin (1890), though they were unable to raise the virulence when it was very low, could raise to some extent the virulence of cultures which caused oedema, but did not kill, by passing through animals together with virulent streptococci. These exalted cultures subsequently retained their virulence. Bardach (1896) succeeded in slightly raising the virulence of a culture, which had become attenuated

[1] In this connection Cobbett says that he has frequently seen similar abscesses form in guinea-pigs treated with large doses of virulent bacilli together with antitoxin, and also in an immunised horse treated with living bacilli. In the latter case the bacilli had retained their virulence.

by two years' growth on artificial media, by numerous passages through dogs, and he states that he was able to raise the virulence for rabbits of bacilli already virulent for these animals by repeated passages through them.

Spronck (1895) found that the virulence of a bacillus which originally killed guinea-pigs in two or three days in a dose of ·2 c.c. could be raised by three passages through these animals to such an extent that a dose of ·1 c.c. killed in 24 hours.

Aronson (1893), Funck (1895) and Martin (1898) all considered that the virulence of diphtheria bacilli which were already virulent could be raised by passages through animals. Ohlmacher (1895, footnote) makes the following observations: "In my own work I have not attempted to recover the bacilli from the seat of inoculation, but believing that the bacilli which gained access to and survived in the visceral organs were the most virulent, I have recovered them from these organs. In this way I was able, for instance, by a single passage through a guinea-pig to make a diphtheria bacillus, which only killed a guinea-pig in 14 days, become so virulent that it killed an animal of equal weight in 30 hours in a much smaller dose." In experiments published later (1902) he states that a short, uniformly staining and slightly virulent bacillus (killing in seven days) after its passage through an animal became long and granular and more virulent.

(c) *Increase of virulence by other means.*

Concetti (1901) states that by anaerobic growth he caused a non-virulent streptothrix-like diphtheria bacillus to become pathogenic and toxic.

Shattock (1898) found that lowly virulent diphtheria bacilli did not acquire virulence when grown in a current of sewer air, even for a period of two months.

(4) *Observations on Changes in Virulence in Diphtheria Bacilli by Transference from one Individual to another.*

The ordinary process of transference of the disease from one individual to another, and the fact that diphtheria bacilli isolated from recent contacts are virulent in 80 % of the specimens tested, prove that in the majority of cases virulent bacilli when transferred retain their virulence. The experimental and natural transference of fibrinous

rhinitis (Chapter VIII) also suggests this view. Under natural conditions, owing to the difficulty of tracing the path of infection, the proof of loss or of increase of virulence, if any occurs, in the passage of a bacillus through a series of individuals is not easy to obtain, unless the type is a particularly distinctive one. In one such instance, in which a very remarkable type infected several individuals in succession in a home for destitute children, Williams (1902, p. 101) observed no change in virulence, although many of the children showed no clinical manifestation of the diphtheria.

Gorham (1901, p. 208) asks "is it not possible that a culture isolated from a healthy throat has lost its virulence for guinea-pigs because of its exposure to the fluids of an immune body and that later, when under the proper conditions in the throat or nose of a susceptible human being, may regain its virulence and be capable of producing clinical diphtheria?" On this subject certain observations have been made from time to time (p. 233), which indicate that non-virulent but otherwise typical diphtheria bacilli do not acquire virulence for guinea-pigs, or give rise to diphtheria when transferred from one individual to another.

In some instances the examination of a person with a slight sore throat, who has not been recently exposed, reveals diphtheria bacilli, which turn out to be non-virulent. In these cases cocci or other organisms are usually also abundant, and probably account for the disease.

(5) *Observations on the Virulence of Diphtheria Bacilli causing Lesions in Different Parts of the same Individual.*

Very few observations have been made on whether diphtheria bacilli, almost certainly derived from the same source, producing diphtheritic lesions in different situations in the same individual, are ever of different types, or show different degrees of virulence. Williams (1902, p. 97) records the examination of two such cases: "The first was a throat case, which had also a vaginal discharge. The same variety of virulent diphtheria bacilli were isolated from cultures from each locality. The second case had throat symptoms and a membrane on a finger wound. In this instance, too, the same variety was isolated from both cultures."

The writer has recently found the same type of fully virulent diphtheria bacillus in the throat and in an ulcer of the foot of a patient, who had been exposed to diphtheria.

(6) *Observations on the Changes in Morphological Type in Diphtheria Bacilli.*

(*a*) *During prolonged persistence in the throat.*

Several observers have stated that they consider that their examinations of convalescent patients lend support to the view that the typical diphtheria bacilli, which are alone found during the acute stages of the disease, gradually lose their characteristics after a more or less prolonged stay in the throat and are finally represented by uniformly staining types, of which Hofmann's bacillus is the furthest removed from the virulent diphtheria bacillus. This view, they say, is also supported by the statistics of the occurrence of Hofmann's bacillus in the throat and nose. According to them Hofmann's bacillus is rarely to be found in clinical cases, but is very frequently present in convalescents, and much more common in those who have been exposed to diphtheria than those who have not. Statistics have already been quoted (p. 205) on the occurrence of Hofmann's bacillus in the throats of contacts and non-contacts, which show great discrepancies amongst the observations of various workers. The general conclusion is, however, that this organism is equally distributed amongst exposed and non-exposed persons in the various grades of the community. The frequency of its occurrence in clinical cases has, however, yet to be dealt with.

Many observers state that it is rarely to be met with in clinical cases, but seldom mention whether their view is based on any other evidence than the examination of cultures from the diseased part. They frequently seem to have lost sight of the fact that, if the swabs are accurately taken from the membrane in clinical cases, few organisms but diphtheria bacilli are likely to be met with in the cultures; even if they were originally present in this situation they would probably be overgrown by the rapidly forming and very numerous colonies of the diphtheria bacillus. The fact of their non-appearance under these conditions cannot, therefore, be taken as proof of the absence of Hofmann's bacilli in other parts of the buccal or nasal cavities.

Further, it is seldom stated how thoroughly the cultures have been searched for colonies of Hofmann's bacillus. If few colonies only are present the probability of overlooking them is very great owing to their small size, and to their similarity to those of the diphtheria bacillus. Smears made from the general surface can be of little value, since the

individual specimens of Hofmann's bacillus are mixed up among the diphtheria bacilli and may easily be overlooked, if few, or mistaken for young specimens of the diphtheria bacillus. A further period of incubation of the cultures often results in the differentiation of the colonies of Hofmann's and diphtheria bacilli sufficient to make an examination at that period of greater value, but such subsequent examinations seldom seem to have been made.

Once the diphtheria bacillus has been found most observers are satisfied to record its presence, and make a diagnosis.

For these reasons the majority of opinions which have been recorded as to the absence of Hofmann's bacillus in clinical cases are of little value.

Observations dealing with the frequent occurrence of Hofmann's bacilli in the throats and noses of convalescents are numerous, but the deductions founded on these observations cannot be regarded as of much value, unless the observers have previously ascertained by adequate experiments whether the bacilli were present in the throat or nose at the height of the disease, and have made a sufficient number of experiments on normal persons of the same class to determine their frequency amongst such persons.

In very few experiments have satisfactory experiments been made on these points.

Hewlett and Knight (1897) state that Hofmann's bacillus replaces the diphtheria bacillus during convalescence, since "almost always the pseudo appears towards the end, the next examination or so revealing an absence of either diphtheria or pseudo-diphtheria bacilli." In order to illustrate their statement they give a list of 24 cases in which the diphtheria bacillus was apparently replaced by Hofmann's bacillus.

Wesbrook, Wilson and McDaniel (1900) also thought that the typical diphtheria bacilli were replaced by uniformly staining forms till finally the D^2 or Hofmann type became the most common or the only type present.

This statement they illustrate by several examples recording the various types found in the course of routine diagnoses of cultures for the establishment and maintenance of quarantine. Cases 1 and 2 showed the following types[1] according to their classification in a series of cultures taken during a period of about three weeks:—

[1] See p. 130, Plate VIII.

Case 1.		Case 2.	
Exam. number	Types present	Exam. number	Types present
1	C, D, E	1	A, C, D
2	A, C, D	2	A, C, D, E
3	A, C, D	3	A, C, D, D^2, E^2
4	A, C, D	4	A^1, C^1, D^1, D^2, E^2, G^2
5	A, C, D, E		
6	A, D, D^1, E, E^2		
7	A, B^1, D, D^1, D^2, E		
8	D^2, D^1		

The authors remark that "these cultures show (*a*) the presence of large granular types, with absence of other types, during the prevalence of clinical symptoms, and (*b*) the entire replacement of granular types by barred and solid types as convalescence was established and just prior to the entire disappearance of all diphtheria-like organisms." They go on to state that "this seems to be the usual order of variation in clinical cases, as has been noted by other observers and frequently in this laboratory." They give also an example in which exactly the opposite conditions were found, and say in regard to it that "such cases in the experience of this laboratory are infrequent, but that they do occasionally occur is beyond doubt."

No. 3.			
Exam. number	Types present	Exam. number	Types present
1	D^2, E^2	4	D, D^2, E^2
2	D^2, E^2, D^1, E^1	5	D, C, A
3	D^2	6	D, C, E, A

They recorded the changes in about 200 cases, and came to the conclusion that the variability of the granular types of bacilli is greater than that of the solid forms.

Gorham (1901) says that in his opinion there seems to be no question that Wesbrook's types, including D^2, are all varieties of the diphtheria bacillus, and considers that during convalescence the typical forms of the diphtheria bacillus become converted into uniformly staining types or Hofmann's bacilli. He asks "Can we not conclude that the granular or barred ($C\,C^1$) are the natural forms of virulent diphtheria bacilli, and that these forms under the influence of body fluids of persons not susceptible to the diphtheria toxin, or who are becoming slowly immune, gradually become non-virulent, and in doing so change to the solid staining types C^2 and D^2?.....If this sequence of form is dependent on the interaction of bacillus and patient, then the same changes of form ought to be shown by a series of examinations of patients who have had clinical diphtheria and have recovered."

To illustrate this change he gives a record of the examinations of 10 cases, all of which at some period show the presence of uniformly staining types.

Williams (1902), on the other hand, made a very careful study of the types found in cultures during successive examinations of 10 cases of diphtheria. "In studying these cases we see that different types appear irregularly throughout the course of the disease, but no sequence can be observed. When pure cultures were isolated from a tube containing these different types it was found that exactly the same variety of bacillus, and only this variety, was obtained as from the earlier culture, showing that the new forms are due, at least in part, to the influence of other bacteria, and it is only when we study a pure culture that we can obtain a true idea of the variety to which the individual bacillus belongs."

"In this series of cases no pseudo forms appeared in the throats throughout the course of the disease that were not there at the beginning." "In these cases the typical diphtheria bacilli from the original nose cultures showed slight differences from those from the throats of the same cases; pure cultures, however, showed that the same variety was present in both throat and nose."

"Some of the pure cultures had such persistent characters that their recognition was particularly easy, and there could have been no question, even to the most superficial observer, in regard to their maintaining the same type throughout the course of the disease."

In conclusion she remarks: "In regard to the appearance of solidly staining pseudo-forms towards the end of diphtheria I would say further that in an examination of hundreds of control smears made in routine work for pronouncing diphtheria cases at the Willard Parker Hospital free from diphtheria bacilli, only occasionally were pseudo-forms observed, and in all of the cases where the typical bacilli persisted for some time and were isolated two and sometimes three times to test their virulence, the same variety continued to be present unmixed with new atypical forms."

As the result of a series of experiments on diphtheria and diphtheria-like bacilli, extending over seven years, this author comes to the conclusion "that not only are there distinct species in this group, but that each species has distinct subspecies or varieties with characteristics which continue to persist under different conditions; these varieties as well as subspecies remain separate, and, when grown under similar conditions, the species show no tendency to become converted into one

another, while the varieties gradually change, approaching a common form. The term variety is applied to a pure culture as a whole and not to individual bacilli, and by it is meant that certain pure cultures possess some persistent morphological and cultural characteristics so different from those of other pure cultures, that the culture has a distinct individuality."

The writer has also frequently noticed in many consecutive examinations for release from quarantine that the same type of bacillus is to be found till its final disappearance. In fact in certain instances, in which the organisms had some marked peculiarity, a glance at a preparation was sufficient to tell from what culture it was obtained. Some of these bacilli were very long, others had an unusual number of stained segments, others large numbers of polar bodies, and a few belonged to the "sheath" variety. A few have been met with persisting for very long periods, which were peculiar in that they produced no visible colonies before 36 hours' incubation. He has also frequently come across cases in which, throughout a long series of examinations, Hofmann's bacilli were never found. In one instance, for example (No. 26, Table, Chapter XII), 50 consecutive examinations were made in 122 days from the throat of a child and Hofmann's bacillus was never encountered. During the whole of this period a morphologically typical diphtheria bacillus was present in the throat. In another case (No. 71) virulent diphtheria bacilli were present for 65 days and ultimately disappeared, but Hofmann's bacillus was never found in 24 examinations. Several other examples may be seen in the table mentioned.

Cobbett (IV. 1901, p. 251) in his records relating to the serial examinations of 11 cases of notified diphtheria, found Hofmann's bacillus at some period in four (36%), and in similar examinations of eight infected contacts found it five times. He also came to the conclusion that Hofmann's bacillus does not replace the diphtheria bacillus.

The statement that Hofmann's bacilli are frequently encountered during convalescence is probably due to the fact that at this period the culture tubes are no longer crowded by innumerable colonies of the diphtheria bacillus, and colonies of other organisms begin to make their appearance. As convalescence advances colonies of the diphtheria bacillus become rarer, and a prolonged search has often to be made. In the course of such examinations colonies of Hofmann's bacillus are met with, and the impression is produced that this organism has replaced the diphtheria bacillus.

According to the statements of several observers the age of the culture, slight differences in the composition of the medium, the presence of contaminating bacteria, and other factors may influence to some extent the type of the bacillus found in the original cultures from the throat or nose. In making any observations on the changes of type some of these factors, especially the possible influence of other bacteria, ought to be eliminated by the preparation of pure cultures.

The important observations of Williams on the constancy of the types in pure cultures prepared from successive cultures from convalescents, from persons infected in two situations, and from persons infected from the same source have already been quoted.

Cobbett (x. 1901, p. 495) also made a similar statement. Some Hofmann's bacilli "as they first appear on the original culture, are far more than others difficult to distinguish on morphological grounds from true diphtheria bacilli, and in not a few instances I have been in doubt until pure cultures have been isolated. However much they may have resembled the diphtheria bacillus at the start, they come in subcultures closely to resemble what I regard as the typical form."

Cobbett's observations on this question the writer has corroborated by a very large number of experiments. Colonies have been very frequently met with, which, together with the typical Hofmann forms, contain varying numbers of segmented bacilli, and occasionally some which consist entirely of segmented bacilli. Such segmented bacilli have a more or less characteristic appearance, and can be differentiated from true diphtheria bacilli by their morphological and staining characters (see p. 200). Pure cultures have been made from such colonies on very many occasions, and also from a very large number of colonies containing a few doubtful bacilli. Almost without exception the young pure cultures have consisted entirely of bacilli of the typical Hofmann form, and in very many cases, in which they were further tested, they produced no acid in glucose broth and no effect on guinea-pigs, even in large doses. According to Wesbrook's method the cultures which have just been mentioned would probably have been recorded as containing type D^2, as well as types D^1 and C^1, and probably in a few cases A^1.

These variations may be to some extent due to the influence of other bacteria or mucus, and are also certainly due sometimes to the composition of the medium, for they have been especially frequently observed in one or two batches made from human serum. The value of the preparation of pure cultures in such cases for diagnostic purposes cannot be over-estimated, and it is also of great importance in

considering the alleged changes of morphological type amongst diphtheria bacilli.

Comparative observations on the occurrence of Hofmann's bacillus in the throats of clinical cases and healthy infected contacts at some period during examinations for release from quarantine, in the throats of contacts not infected with diphtheria bacilli, and of non-exposed persons belonging to the same class of the community, have seldom been made during an outbreak. Observations of this character are, however, more likely to give to the observer a true idea of the distribution of Hofmann's bacillus and its connection or lack of connection with the diphtheria bacillus than any number of repeated examinations of cultures from convalescents alone.

Cobbett (1901) records during one outbreak the results of consecutive examinations of 18 persons, who either suffered from clinical diphtheria, or harboured morphologically typical diphtheria bacilli, and amongst these nine (50%) at some period showed Hofmann's bacilli. Amongst 650 other persons examined, some of whom had not been exposed, Hofmann's bacilli were found in 157 (24·1%). Although the precise figures are not given the author states that "it was certainly not found more frequently among those who had come into contact with diphtheria than those who had not. It was less frequently found among the scholars of the 'higher grade' school, where there was much diphtheria, than among the scholars of the ordinary schools, where there was little or none. Among the small number of children of the upper class examined it was conspicuous by its rarity. When it is remembered that the infected persons were examined on the average six times each and some only showed a few colonies at one examination, it seems probable that had the healthy persons been examined as often the incidence of this organism amongst them might have been as high as among the infected."

In another outbreak (x. 1901), investigated several months later, he confirmed the opinions he had previously formed.

Graham-Smith (1904, p. 297), in an extensive outbreak at Cambridge, found that the proportion of persons infected with Hofmann's bacillus was nearly the same among convalescents recovering from diphtheria (48%), contacts infected with diphtheria bacilli (51·9%), non-infected contacts (43·7%), and non-contacts (54·2%).

Nearly all these persons were children belonging to the same social class. Amongst adults, whether infected with diphtheria bacilli or not, the percentage of infection with Hofmann's bacilli was much smaller.

During a previous outbreak at Colchester (Graham-Smith, 1903) 112 persons, notified as suffering from diphtheria, were examined on the average six times each for release from isolation. Some of these were first examined after the disappearance of the diphtheria bacillus. Hofmann's bacillus was at some time present in cultures from 62 (55·3%) of them. Amongst all the persons examined in the town, whether exposed or not, it was present in 31%, but 63% of the scholars of the public elementary schools, amongst whom most of the notified cases of diphtheria occurred, harboured this organism. On further analysing these records, however, it was found that the percentage infected with Hofmann's bacillus was the same amongst scholars attending schools in which diphtheria was common as amongst those attending schools in which few or no cases occurred.

The records of this outbreak agree therefore with those of the one just quoted in showing that Hofmann's bacillus is as common amongst normal school children attending the public elementary schools as amongst others who are recovering from diphtheria or have been exposed to it.

(*b*) *By transference from one individual to another.*

Many instances are to be found recorded in the literature of patients infected from the same source harbouring bacilli similar on culture. The writer has frequently noticed the same type of diphtheria bacillus in all members of an infected family, and in one instance of a small outbreak in a village found that every infected person, whether showing clinical signs or not, harboured very long bacilli with many segments. Williams (1902) records some observations in an outbreak in a home for destitute children bearing on this point. Two deaths had occurred in this institution, but the type of bacillus had not been recorded. Later an outbreak of sore throats began, and cultures from all cases were found to contain the same variety of virulent bacillus as the first case. The variety was peculiar in that many coccus-like forms appeared when the organisms were grown on agar. It was also peculiar in producing very delicate colonies on agar, and a very scanty growth in broth. Observations such as these show that changes in morphological type do not occur when diphtheria bacilli are transferred from one individual to another.

(*c*) *By growth on artificial media.*

It has already been shown that both diphtheria and Hofmann's bacilli exhibit differences in morphology more or less great when

grown on different media. When, however, the organisms are again sown on serum the original type appears. The question here to be discussed is, whether by means of growth on artificial media the characters of any given race of bacilli can be so altered that when grown on serum at 37° C. its morphological appearances are markedly affected. It seems to be universally accepted that by more or less prolonged growth slight changes in morphology are brought about. The experiments of Hewlett and Knight would lead us to suppose that by prolonged cultivation alone the morphological type and even biological character of diphtheria-like bacilli can be profoundly altered. The observations of several other observers have caused them to believe that the morphological types of diphtheria bacilli are unstable and can readily change in culture. Wesbrook, Wilson and McDaniel (1900), for example, state that "attempts to classify types of bacilli on the appearance of microscopical fields of presumably pure cultures had to be abandoned," and Ohlmacher (1902) believes that most of the common characteristics of the diphtheria bacillus are unstable.

The former observers give several examples, some of which are quoted below, of the change of form in pure cultures. "When the observations here recorded were begun, the stocks had been isolated by a repetition of the process of colony picking and subsequent streaking out from three to fifteen times. Each subculture having been made in the same way, it is apparent that the stocks at the time of the last observation had been subjected to this purification process from twenty to forty or more times."

Pure stock No. 2 (clinical case).

No. of culture		Morphological types in 16—18 hour cultures
1.	Original throat culture	C, D
2.	Pure culture*	B, C^1, C, D^2
3.	,, ,,	D, E, B, D^2
4.	,, ,,	B, C^1
5.	,, ,,	A, D, E
6.	,, ,,	B, A, C, C^1
7.	,, ,,	B, C, A, C^1
8.	,, ,,	A, C, E

* Two years after isolation.

Pure stock No. 5 (non-clinical case).

No. of culture		Morphological types in 16—18 hour cultures
1.	Original throat culture	C, D, E^2
2.	Pure culture†	B, C, C^1
3.	,, ,,	C, D, E, E^2
4.	,, ,,	A, B, C, D, E, E^2
5.	,, ,,	A, C
6.	,, ,,	A, C, D
7.	,, ,,	C^1, B, A
8.	,, ,,	C, D, C^1, A

† Eight months after isolation.

In stock No. 2 the general tendency is to the predominance of the long forms throughout. The culture remained virulent for guinea-pigs throughout its whole history. In stock No. 5, which was obtained from a child who had not recently been exposed to diphtheria, the large

granular types predominate, but it was never possible to kill a guinea-pig even with large doses.

Pure stock No. 6 (clinical case).

No. of culture		Morphological types in 16—18 hour cultures
1.	Original throat culture	D^2
2.	Pure culture	D^2
3.	,, ,,	E^2, D^2
4.	,, ,,	G^2
5.	,, ,,	E^2, G^2
6.	,, ,,	E^2, D^2
7.	,, ,,	E^2, G^2
8.	,, ,,	D^2

This stock, although there were never any other than solid types present, was apparently a true diphtheria bacillus, and killed guinea-pigs not protected with antitoxin.

Hadley (1907) considers that his "observations permit of little doubt that a single morphological variety of the diphtheria organism is decidedly modifiable; and that not only may the granular types of the organism be resolved into the solid staining forms, but the opposite may also be true."

In opposition to these views are those put forward by certain other observers, who believe that certain types are very stable and that pure cultures of them possess some persistent morphological character. Williams (1902) kept pure cultures of a large number of diphtheria bacilli on agar at 36° C. transplanted every two to four weeks, and 10 cultures of Hofmann's bacilli for the same time in broth and on serum. At the end of that time when transplanted upon other culture media the individual characteristics of the cultures were unchanged. Also several different varieties of diphtheria-like bacilli were kept on media for six months to a year, and all retained the characteristics of the original cultures. Certain diphtheria bacilli with peculiar characters already described (p. 249), obtained from numerous individuals in an outbreak at a school for destitute children, were observed through numerous generations for eight months, but showed no changes in morphology. Williams concludes from her observations that pure cultures of diphtheria bacilli continue to show the same characters through many culture generations and that there are many distinct varieties of diphtheria-like bacilli, all of which in serial pure cultures retain the characters of the original culture. The writer has also often noticed that pure cultures of diphtheria bacilli kept growing on serum for numerous generations continue to show the same general morphological

characters as the original cultures. He has also found that diphtheria bacilli grown for 10 days at 37° C. in media containing different sugars and carbohydrates, when transplanted on to serum, showed the same morphological peculiarities as the original cultures.

(*d*) *By passage through animals.*

By some observers certain changes in morphological type have been noted in bacilli recovered from the tissues of animals. Hadley (1907) found that a virulent D^2 type, when recovered from the tissues of a guinea-pig, became barred. On the other hand three strains, two showing CC^1 types and one C^1C^2 types, after recovery only showed D^2 type. The most remarkable examples have been quoted by Ohlmacher (1902). He experimented with three organisms, and concluded that by a short sojourn in an immune animal a diphtheria may be converted into a Hofmann-like bacillus, and that the reverse may be brought about by passing the organism through a susceptible animal. He states that a long granular diphtheria bacillus after recovery from the subcutaneous tissue of a rat became short and uniformly staining, that a uniformly staining, but pathogenic, bacillus after recovery from the spleen of a guinea-pig became granular, and a short uniformly staining Hofmann-like bacillus after its passage through a guinea-pig became long and granular. The statements of Ohlmacher cannot be accepted as universally true, as Williams (1902), experimenting on four varieties of diphtheria bacilli, found no changes in morphological type after they had been in the body of an immune host (white rat) for 48 hours. The same observer also found no change in morphological type in non-virulent diphtheria bacilli after successive intraperitoneal inoculations through guinea-pigs. The writer has recently experimented with 10 strains of diphtheria bacilli, five virulent and five non-virulent. These have been recovered after two to five days' sojourn in the subcutaneous tissues of white rats, but no differences in morphology were noted. In two cases small abscesses had formed, and these also contained bacilli of the same type as those injected.

(7) *Observations on the Pathogenicity of Hofmann's Bacillus.*

If, as has frequently been asserted, Hofmann's bacillus is merely an attenuated and slightly altered diphtheria bacillus, capable under certain conditions of regaining its virulence and giving rise to diph-

theria, it might be expected that in the process of attenuation before complete loss of virulence had been attained, or on the other hand during the process of regaining pathogenic power before sufficient virulence had been acquired to produce typical diphtheritic lesions, the organism would be capable of setting up some disease in man, and giving rise to lesions in guinea-pigs, animals peculiarly susceptible to diphtheritic poison.

Observations dealing with these points, so far as they bear on the relationship of diphtheria and Hofmann's bacilli, are considered under the following headings: (*a*) Observations on Hofmann's bacilli apparently pathogenic to man. (*b*) The virulence towards guinea-pigs of Hofmann's bacilli recently isolated from (1) healthy persons, (2) persons suffering from diphtheria, (3) convalescents from diphtheria, (4) healthy persons infected with diphtheria bacilli, (5) healthy members of infected families not themselves infected with diphtheria bacilli, (6) persons suffering from throat lesions more or less resembling diphtheria.

(*a*) *Observations on Hofmann's bacilli apparently virulent to man.*

Many observers are of the opinion that Hofmann's bacillus is capable of giving rise to disease in man. The opinions, however, vary as to the real nature of such conditions. While some consider that the disease is in reality mild diphtheria, others think that it is a specific affection produced by the Hofmann's bacillus. Others again, while admitting that this organism is not infrequently encountered in diseased conditions, consider that it is not in any way related to them.

Hewlett and Knight (1897) quote several histories to show that Hofmann's bacillus is associated with mild anginas, which are free from complications, end in recovery, and are not followed by sequelae. Richmond and Salter (1898) found it to be the only organism present in an outbreak of post-scarlatinal diphtheria in a hospital ward, and Priestly (quoted by Caijer, 1904) records an outbreak of throat-illness in a school at Lambeth characterised by tonsillar exudation, in which the Hofmann's bacillus and no other was present in 38 out of 43 of those attacked. These are only examples of records of a similar nature, many of which are to be found in the literature. It will be pointed out later (Chapter XI), however, that diseases simulating diphtheria occur, the causal agents of which do not grow on the ordinary media under the

usual conditions. The presence of Hofmann's bacillus in culture does not therefore necessarily prove, unless such other organisms have been excluded, that it is the causal agent. Further, even if organisms resembling Hofmann's bacillus in morphology are shown to be the only ones present in large numbers, their cultural and other characters must be worked out before their identity can be established.

Wesbrook, Wilson, and McDaniel (1900), for instance, found in an outbreak of diphtheria at Owatonna that the type of diphtheria bacillus most frequently present closely resembled the Hofmann's bacillus. It could, however, be differentiated from the latter by the fact that it formed acid in broth, and a strong toxin, whose effects could be neutralised by antitoxin. They also seem to have encountered the same type of diphtheria bacillus on other occasions.

Ruediger (1903) and later Hamilton (1904) have also recorded the presence in severe throat affections of organisms, apparently belonging to different species, which, although they somewhat resembled Hofmann's bacillus in morphology, differed in their cultural and pathogenic properties (Chapter X).

The evidence supporting the view that Hofmann's bacillus is capable of producing disease in man is almost entirely based on the presence in cultures from the throat of organisms morphologically identical with this bacillus. Most observers have only examined young serum cultures and have not excluded the possibility of the lesions being due to other organisms, which either grow slowly on serum or refuse to grow on it, or to other organisms, such as *B. fusiformis*, which cannot be cultivated aerobically, and only with difficulty in anaerobic cultures. Bearing in mind the extremely common occurrence of Hofmann's bacillus in the throats and noses of healthy persons, and the fact that this organism is non-pathogenic to guinea-pigs, animals peculiarly susceptible to the products of the diphtheria bacillus, observations of a more convincing nature, which exclude the presence of all other known pathogenic organisms, are needed before its capacity for producing disease in man can be regarded as proved.

(*b*) *The virulence towards guinea-pigs of Hofmann's bacilli recently isolated from*

(1) *Healthy persons.*

Gorham (1901, p. 209) says that "type D_2, frequently called the atypical form of *B. diphtheriae*, is probably to be included in the 'pseudo-diphtheria' or 'Hofmann' group of many writers. It has been

shown to be frequently pathogenic to guinea-pigs, and, when so, protection is afforded by the use of commercial antitoxin."

The Massachusetts Committee (1902, p. 18) in commenting on this question make the following statement: "Both these observers (Gorham and Wesbrook) several times isolated solid forms in pure culture from healthy persons, and proved them virulent for guinea-pigs. Such a condition, however, must be very rare, as no other members of the Committee have ever found these forms present alone in clinical diphtheria; and when isolated by them from healthy persons, they have always proved to be non-virulent."

The experiences of almost all other observers are in agreement with this statement.

Park (1898), although he has apparently met with two cultures of the "pseudo type" which were virulent, states (1900, p. 351) that Hofmann's bacilli "never produce diphtheria toxin," and "that no facts have come to light which indicate that bacilli, which do not produce diphtheria toxin in animals, ever produce it in man."

Amongst those who have made the most numerous experiments are Cobbett and Neumann. Cobbett (4. x. 1901) isolated and tested 86 strains, and found them all to be non-virulent in doses of 2 c.c. of 48 hour broth cultures, and Neumann (1902) inoculated into guinea-pigs in doses of 2 c.c. of 48 hour broth culture 78 strains obtained from normal and diseased noses without any effect.

During the course of the last five years the writer has isolated and tested on guinea-pigs a large number of bacilli obtained from healthy persons, which though they differed slightly from typical Hofmann's bacilli in the original cultures, resembled them in sub-cultures in every morphological and cultural character, including the production of an alkaline reaction in glucose broth. All without exception proved non-virulent to guinea-pigs.

(2) *Persons suffering from diphtheria.*

Cobbett (iv. 1901) isolated in at least two cases both Hofmann's bacilli and diphtheria bacilli from original serum cultures obtained from patients suffering from clinical diphtheria, and proved the former organisms to be non-virulent. In a few instances the writer (Graham-Smith, 1904, p. 291) has also isolated Hofmann's bacilli from the original serum cultures taken from clinical cases of diphtheria at the height of the disease[1]. In all these cases the bacilli have produced

[1] Other details concerning some of these cases are given in Chapter XII. (Table. Nos. 11, 12, 24, 39, 54, 88.)

an alkaline reaction in glucose broth, and doses of 2 c.c. of well-grown 48 hour broth cultures have been without effect on guinea-pigs. On the other hand the diphtheria bacilli isolated from the same cultures have been fully virulent. Petrie (1905) also isolated three races from patients suffering from diphtheria and found them to be typical in all respects, and totally innocuous to guinea-pigs.

(3) *Convalescents from diphtheria.*

Cobbett (IV. 1904) isolated Hofmann's bacilli from the throats of three convalescents when diphtheria bacilli were still present, and found them to be typical in all respects and totally without virulence for guinea-pigs, and Williams (1902) made similar observations. On several occasions (six) the writer has also isolated Hofmann's bacilli from the throats of convalescents at an early stage, and has never found them to produce any greater effects than a transient oedema in guinea-pigs.

(4) *Healthy persons infected with diphtheria bacilli (infected contacts).*

As in the case of patients suffering from diphtheria and convalescents, the writer has isolated and tested a number of strains of Hofmann's bacilli from healthy persons infected with virulent or non-virulent diphtheria bacilli, and has always found them to be non-pathogenic to guinea-pigs. Cobbett (IV. 1901, p. 249) also isolated Hofmann's bacilli from the throats of infected contacts and proved them to be non-virulent, and similar observations have been made by a number of other workers.

(5) *Healthy members of infected families not themselves infected with diphtheria bacilli.*

Hofmann's bacilli, isolated from healthy non-infected members of families in which diphtheria had recently occurred, have been shown by several observers to be totally non-virulent for guinea-pigs.

(6) *Persons suffering from throat lesions more or less resembling diphtheria.*

Cobbett (IV. 1901) reports two instances in which Hofmann's bacillus was the only diphtheroid organism isolated from cultures taken from persons clinically suffering from diphtheria. In neither case did the organisms show any indications of virulence towards guinea-pigs, although 2 c.c. of three, four, and seven day old broth cultures were injected. Petrie (1905) isolated two strains from suspected cases of

diphtheria and found them to be typical Hofmann's bacilli in all respects, and innocuous to guinea-pigs.

For some time past the writer has isolated and tested on animals all examples of Hofmann's bacillus obtained from persons suffering from lesions resembling diphtheria sufficiently closely to make a bacteriological examination desirable, and in whom no diphtheria bacilli have been found. Twenty-six such strains have been inoculated into guinea-pigs without producing any results. Smears made direct from the swab were examined in a number of these cases, some of which showed fusiform bacilli and spirilla, and some large numbers of cocci, etc. It is possible that in some of these cases a multiplication of the Hofmann's bacilli present in the normal condition takes place secondary to the inflammation set up by the other organisms, since very large numbers of colonies are sometimes found. In other cases only a few colonies of this organism develop, not more numerous than are frequently to be obtained from the normal throat. No experiments appear to have been made with the view of ascertaining whether Hofmann's bacilli, frequently present in the normal throat, multiply during inflammatory processes due to other organisms. Nevertheless, it is a matter of some interest in forming an opinion in regard to the causal connection between Hofmann's bacilli and some of the conditions in which they are found. Whether or not such a multiplication is proved to occur, it must be borne in mind that innumerable colonies of Hofmann's bacilli can frequently be obtained from the healthy nose.

Summary of Section 7.

The observations which have been quoted in this section show that diphtheria bacilli, which in other respects are typical of the species, may in very rare instances morphologically resemble Hofmann's bacilli, and that other pathogenic species more or less resembling Hofmann's bacilli, but easily distinguishable by their cultural characters, may produce lesions in man. These facts are in no way opposed to the general conclusion from the other facts recorded, that morphologically typical Hofmann's bacilli which are culturally characteristic producing no acid in glucose broth, show (according to almost all observers) no virulence to guinea-pigs even when inoculated in large doses.

If Hofmann's bacillus is merely an attenuated form of the diphtheria bacillus, incapable of producing lesions in guinea-pigs when cultivated from the throats and noses of healthy persons, specimens isolated from

persons suffering from diphtheria, convalescents, and infected contacts should exhibit some signs of virulence towards guinea-pigs when injected in comparatively large doses. Again, if, as Gorham appears to think possible, the fluids of an immune person are capable of changing a virulent diphtheria bacillus into a Hofmann's bacillus shortly after infection, some of the latter, when discovered in the mouths of healthy members of infected families, and therefore according to this view probably recently converted diphtheria bacilli, ought to show traces of virulence. Under none of these conditions, however, is any trace of virulence shown. Finally this bacillus, when discovered as the only diphtheroid organism in mild, or even severe, throat or nose lesions is without virulence for guinea-pigs. Most of such diseased conditions are probably due to other bacteria, and there is little evidence in support of the view that Hofmann's bacillus is even capable of producing non-diphtheritic inflammatory lesions in man. In fact the weight of the evidence, so far as guinea-pig experiments are of value, points to the conclusion that under all conditions Hofmann's bacillus is a harmless saprophyte.

(8) *Attempts to make Hofmann's Bacillus virulent for Guinea-pigs.*

(*a*) *By passage through animals.*

An account of the experiments which have been made with the purpose of attempting to convert Hofmann's bacilli into diphtheria bacilli by means of passages through birds or otherwise has already been given (p. 219).

Apart from these experiments, several observers have tried to impart virulence to Hofmann's bacillus by passages through animals. From one of Ohlmacher's experiments it might be concluded that Hofmann's bacillus could be rendered virulent by a single passage through a susceptible animal, for example a guinea-pig, but the experiments of Bergey (1898, p. 46), Williams (1902), and others, distinctly show that even by successive passages through guinea-pigs Hofmann's bacilli cannot be rendered virulent to these animals. A passage through an immune animal, such as a rat, is also apparently incapable of producing virulence in this organism. The writer recently injected four strains of Hofmann's bacillus subcutaneously into white rats, and recovered them from the site of inoculation after 48 hours. The rats were unaffected and the cultures made from the organs

remained sterile. Attempts to recover one strain after three days was unsuccessful. All the recovered strains were totally non-virulent to guinea-pigs in doses of 3 c.c. of 48 hour broth cultures. Two of these strains had been obtained from persons suffering from diphtheria-like diseases of the throat.

Fibiger (1895) was unable to raise their virulence by inoculation into guinea-pigs together with virulent streptococci.

(*b*) *By growth with other organisms.*

Bernheim (1894) was unable to make a Hofmann's bacillus virulent by continued cultivation together with streptococci.

(9) *Observations on the Changes in Morphological Type of Hofmann's Bacilli.*

(*a*) *After passage through animals.*

Films made from the seat of inoculation often contain many bacilli whose morphological characters do not entirely correspond with those of the organisms in the cultures from which the inoculation was made. In cultures made from the seat of inoculation, however, it is usually found that the organisms which grow are all of the same general morphological type as those in the original culture. Ohlmacher (1902) states that in one experiment a short, uniformly staining, Hofmann-like bacillus, after a passage through a guinea-pig, completely altered its morphological character in culture and became long and granular, and Hadley (1907) describes a D^2 type which "killed in 39 hours, with all the usual signs of diphtherial poisoning. When the organism was recovered it was found to be a barred variety." Williams (1902) on the other hand observed no changes in morphology in Hofmann's bacilli in cultures recovered from guinea-pigs after several successive intra-peritoneal passages through these animals, nor were any changes found after a sojourn of 48 hours in the body of an immune host (rat).

In a few experiments recently made with Hofmann's bacilli, obtained either from clinical cases of diphtheria or persons suffering from diphtheria-like diseases, the writer noticed no morphological changes in the cultures recovered from the tissues of inoculated rats.

(*b*) *In cultures.*

No observer appears to have noticed any changes in morphological type in Hofmann's bacilli grown on various culture media for considerable periods of time and then recultivated on serum. When grown

on broth, media containing sugars, hard-boiled egg, and various other substances long, and even segmented, or very short types may be seen after a few days' cultivation. Transference of the organisms from such media on to serum invariably results in the culture exhibiting the ordinary Hofmann type, showing that no real change of type has occurred under these circumstances.

(10) *Evidence bearing on the Relationship of Diphtheria and Hofmann's Bacilli derived from Preventive Measures.*

A number of outbreaks of diphtheria, both limited and extensive, have now been recorded in which the preventive measures have included the examination of contacts and other possible carriers of the disease, and the isolation of such as have been found infected with diphtheria bacilli. In several instances (Chapter XII) these measures have been highly efficacious and have resulted in the complete, or almost complete, cessation of the disease, although persons harbouring Hofmann's bacilli have not been isolated or treated in any way. At the times when diphtheria is epidemic the conditions should prevail, if there are any, which are capable of turning Hofmann's bacilli into diphtheria bacilli. Yet in some of these outbreaks numbers of children harbouring Hofmann's bacilli, when separated from those harbouring diphtheria bacilli, have remained perfectly well without any cases of diphtheria amongst them. Even Wesbrook (1905), who in one outbreak met with diphtheria bacilli morphologically resembling Hofmann's bacilli, states in his most recent publications that in dealing with outbreaks the bearers of all other forms of diphtheroid bacilli than types A, C, D may be safely neglected.

These facts, so far as they go, indicate that Hofmann's bacilli under conditions which seem most suitable for the exaltation of their virulence, if they are really attenuated diphtheria bacilli, are incapable of giving rise to diphtheria.

The Opinions of recent Investigators on the Relationship of the Diphtheria to the Hofmann's Bacillus.

In the previous sections the various observations bearing more or less directly on the relationship of the diphtheria to the Hofmann's bacillus have been quoted, and in this section the views of some of

those who have worked at the subject within the last ten years[1] are given. Some of these observers have investigated the problem from several points of view, whilst others have made extensive inquiries into certain points which appeared to them of fundamental importance. Others have expressed opinions on the most superficial observations. The views of the latter have not been recorded.

Those who consider that by artificial means they have succeeded in converting one organism into the other naturally believe in their identity. Of these Hewlett and Knight (1897) and Richmond and Salter (1898) are the most prominent. On various grounds, including experiments and observations on successive cultures from patients, Simonin and Benoist (1898), Lesieur (1901), Levin (1901), Gorham (1901), Ohlmacher (1902), and Behring all regard these organisms as very closely connected, if not identical. Salus (1902) regarded Hofmann's bacillus as a diphtheria bacillus which had become saprophytic, but considered that no diagnosis of diphtheria should be given when it was found. Wesbrook (1900, 1905) seems to regard all of his types as variants of the diphtheria bacillus, but, nevertheless, considers that in dealing with epidemics the Hofmann type may be safely neglected.

On the other hand a large number of observers have either definitely expressed the opinion that the organisms are distinct species, or that, whatever their relationship, Hofmann's bacillus has no etiological significance in the causation of diphtheria. Others again have only investigated points of special importance in regard to relationship,

[1] The authority of Roux (1890), whose opinion justly carries great weight, has often been quoted in support of the idea that Hofmann's bacillus is related to the diphtheria bacillus. But this is not right, for his remarks on the pseudo-diphtheria bacillus were made in comparatively early days, when the importance of acid-production had not been generally recognised, and before Hofmann's bacillus had been clearly distinguished from the so-called non-virulent diphtheria bacillus. Indeed in speaking of the relationship of what he calls the pseudo-diphtheria bacillus to the true diphtheria bacillus, Roux distinctly said that the former produced acid. He, therefore, at that moment was not speaking of Hofmann's bacillus, which forms no acid, but of what is generally now called the non-virulent diphtheria bacillus. It is only when he goes on to speak of the great frequency of the pseudo-diphtheria bacillus, both in Paris and in a country village, that one believes that he must have been then referring to Hofmann's bacillus, for the non-virulent diphtheria bacillus is relatively uncommon. It is highly probable that he did not test the acid-production of all these bacilli, the importance of the test then not being recognised, and that he confused Hofmann's bacillus, which forms no acid out of glucose, and is a common inhabitant of the throat, with the much rarer acid-forming non-virulent bacillus which cannot be distinguished by its appearance from the true diphtheria bacillus, and included them both in the term pseudo-diphtheria bacillus.

and have shown that no evidence could be obtained in favour of any connection between the organisms. Amongst such observers are the following, none of whom appears to attach any importance to the finding of Hofmann's bacillus in cultures: Peters (1896), Neisser (1896), Prochaska (1896), Fraenkel (1897), Spronck (1897), Glucksmann (1897), Todd (1898), Franke (1898), Bergey (1898), Theobald Smith (1898), Preisich (1899), Garratt and Washbourn (1899), Lubowski (1900), Gromakowsky (1900), Cobbett (1901), Zupnik (1902), Williams (1902), Schabad (1902), Neumann (1902), Korchoune (1902), Gordon (1902), Schwoner (1903), Thomas (1904), Graham-Smith (1904), Lewandowsky (1904), Petrie (1905).

Some of these investigators have expressed the opinion that Hofmann's bacillus is frequently associated with an uncleanly condition of the mouth and teeth, and its more frequent occurrence amongst the children of the lower classes lends support to this view.

A number of workers have expressed themselves as unconvinced by the arguments brought forward on one side or the other, and consider that the controversy cannot yet be regarded as settled.

Summary of Chapter VI.

Hewlett and Knight (1897) considered that by heating cultures of the diphtheria bacillus they had once succeeded in converting it into a Hofmann's bacillus, but failed to bring about the transformation on other occasions. Other subsequent observers have also failed to confirm their statements, and even Hewlett (1904) has recently been unable to succeed. Trumpp (1896) also thought that he had brought about the same transformation by successive passages through guinea-pigs together with a non-fatal dose of diphtheria toxin. Hewlett and Knight (1897) as well as Richmond and Salter (1898) described experiments in which Hofmann's bacilli were apparently converted into diphtheria bacilli, the former observers by prolonged cultivation, the latter by repeated passages through small birds. Neither of these experiments appears to have been successfully repeated, and even the virulence of Hofmann's bacillus for the goldfinch has been denied.

In view of the fact that these experiments were made several years ago, and have not been successfully repeated, and of the fact that much evidence has accumulated against the identity of the two organisms, great importance cannot be attached to these experiments, unless they can be confirmed by subsequent experimenters.

The results of the most recent and exhaustive experiments have shown that Hofmann's bacillus produces no toxoids in culture, and moreover animals cannot be immunised against diphtheria bacilli by successive inoculations with cultures of this bacillus.

Again the capacity for producing acid out of certain sugars is confined to the diphtheria bacillus, Hofmann's bacillus being incapable of producing acid out of any of the substances which have been tested.

The evidence of agglutination experiments also decidedly points against the connection of the two species.

The general conclusion from these experiments, which have the most direct bearing on the relationship of the two organisms, is that no sufficient evidence has been produced in favour of their identity. In fact the most recent observations show, so far as they can, that these bacilli belong to distinct species.

Very numerous other observations and experiments have been made which have some bearing on this question.

Experiments have been quoted which prove that the majority of the morphologically typical diphtheria bacilli, which are found in healthy persons exposed to diphtheria, are fully virulent, and that they retain their virulence for long periods; in many instances up to the time of their final disappearance. Very little evidence can be produced for the statement, which is so frequently made, that during prolonged persistence in the throat diphtheria bacilli gradually lose their virulence. Experimental attempts to artificially decrease the virulence of diphtheria bacilli produce somewhat conflicting evidence, but show that under any conditions their attenuation is a matter of difficulty and uncertainty. Attempts to give virulence to totally non-pathogenic diphtheria bacilli have been almost uniformly unsuccessful, and even attempts to raise the virulence of lowly virulent bacilli have frequently failed. There is also no evidence to show that the virulence of diphtheria bacilli is altered by transference from one individual to another, or when different parts of the same individual are affected. Whatever the final verdict may be as to the relationship of the virulent diphtheria bacillus to the non-virulent form, no inferences can be made from it as to the relationship of the diphtheria bacillus to the Hofmann's bacillus.

It has frequently been asserted that diphtheria bacilli during convalescence gradually change their morphological type and become converted into Hofmann's bacilli, but many of the investigations on this subject are for various reasons of doubtful value, and the assertion has certainly not been conclusively proved. In fact there is much evidence

to show that such a change does not take place. Changes of morphological type do not seem to occur by transference from one individual to another.

According to some observers prolonged cultivation is apt to change the morphological type, according to others it is not. In regard to changes of morphological type by passages through animals the statements are conflicting.

On the whole these experiments and observations also point to the conclusion that diphtheria and Hofmann's bacilli are not related to one another.

It has been asserted on various occasions that Hofmann's bacilli may be pathogenic to man, but no bacteriological proof has been produced, and, except by one or two observers, Hofmann's bacilli derived from all sources have been found to be non-virulent to guinea-pigs in fairly large doses. Attempts to give virulence to this organism or to change its morphological type have mostly been unsuccessful. Further the evidence derived from the bacteriological control of outbreaks of diphtheria, and the fact of the very general distribution of this organism are against the etiological significance of Hofmann's bacillus in diphtheria. Finally most modern investigators appear to regard diphtheria and Hofmann's bacilli as belonging to different species.

An examination of even the most recent writings on the bacteriology of diphtheria shows that many workers are still content to found far-reaching conclusions on the morphology in culture alone. Some have not even gone so far but have based their statements on the direct examination of smears. In not a few of these papers it is evident that the writers are scarcely aware that the identification of bacteria depends on anything else than their morphological characters, and such important aids to classification as their action on sugars and the effects of animal inoculations are often not even mentioned. In fact it is not too much to say that much of the work that is published on problems apart from routine diagnosis is absolutely valueless. Some of the conclusions may be perfectly correct, but the statements are advanced without proofs, such as would be required in any other branch of bacteriology. Inquiries which have for their subjects such important questions as the occurrence of diphtheria bacilli in persons not recently exposed to diphtheria, their occurrence in apparently non-diphtheritic lesions, in unusual situations, in milk, animals, food-substances, dust or soil, require that the identity of the bacilli should be proved by every possible means, including the inoculation of guinea-pigs, both with and

without antitoxin. Yet on all these subjects papers have been written without the mention of any experiments to confirm the diagnoses founded on morphology. On such points as these the unconfirmed morphological diagnosis of even the most experienced observers cannot be accepted as proof of identity.

Whilst it is highly desirable that those who make routine bacteriological diagnoses should from time to time test the accuracy of their opinions founded on morphology by isolating and thoroughly examining bacilli from doubtful cultures, the necessity for this procedure is not so great as in work which is of the nature of research. In the former case no doubt a few patients suffer from inaccurate diagnoses, and the errors of the observer are likely to become more numerous as time goes on, but in the latter persons unacquainted with bacteriological methods are apt to accept unjustifiable conclusions on the prevalence of diphtheria bacilli to the great detriment of bacteriological preventive measures.

Even when every means of identification have been used by practised observers conflicting statements are common as exemplified in this chapter. Many of these are probably due to the unfortunate confusion which has prevailed owing to the wide range covered by the term "pseudo-diphtheria" bacillus.

There can be no doubt that Hofmann's bacillus is a common inhabitant of the throats and noses of healthy persons, at any rate in this country. Moreover numerous experiments have clearly demonstrated that from whatever source they are derived they are non-pathogenic for guinea-pigs in ordinary doses. Further evidence is rapidly accumulating to show that many other species of diphtheria-like organisms are to be found in the throat, nose, eye, genital tract, and on the skin of healthy and diseased persons, some of which more closely resemble the diphtheria bacillus and others the Hofmann's bacillus in morphology. "Since there are so many different forms or varieties of diphtheria-like bacilli it is quite possible that some of them are so closely related to the diphtheria bacillus that under certain conditions they readily develop its characteristics. This seems to be the only way to explain the apparent discrepancies in the results obtained by different observers" (Williams, 1902, p. 107). Such closely related varieties, if they exist, appear to be very rare.

Whatever the ultimate conclusion on this point may be, it is evident that much of the work dealt with in almost every section of this chapter might be advantageously repeated and extended. Opportunities constantly occur to those who are engaged in this subject of testing the

accuracy of some of the observations. Many of these points are best worked out by very thorough experiments on a small scale, including all the necessary procedures for testing virulence under constant conditions, acid production, etc. In the case of inquiries into the change of type during long persistence in the throat or nose the influence of contaminating bacteria and other disturbing factors ought to be eliminated by the preparation of pure cultures.

Even if it is eventually proved, contrary to the opinion here expressed, that certain strains of Hofmann's bacilli represent saprophytic diphtheria bacilli, capable under artificial conditions of regaining virulence, this fact would not materially affect the conduct of bacteriological preventive measures unless proof was forthcoming that the change could also occur under natural conditions.

CHAPTER VII.

EXPERIMENTAL AND NATURAL DIPHTHERIA IN ANIMALS.

The lesions of experimental diphtheria in guinea-pigs, rabbits, dogs, rats and mice. Experimental and natural infection in cats, cows and horses. Diphtheria bacilli in the blood and organs of experimental animals. Diphtheria-like bacilli found in animals. Experimental lesions in birds. Observations on the relationship of avian to human diphtheria. Holmes' observations. Summary.

I. The Lesions of Experimental Diphtheria in Guinea-pigs.

(*a*) *Macroscopic Lesions.*

Subcutaneous inoculations of pure cultures of diphtheria bacilli into the abdominal walls of guinea-pigs cause death usually within three days, and in typical cases result in the following gross anatomical lesions.

On removing the skin a grayish, necrotic, membranous focus is found at the seat of inoculation, frequently surrounded by a red zone of varying size. Underlying this area the abdominal wall is greatly congested. The subcutaneous tissue on the inoculated side is oedematous. The oedema may be slight, limited to a small area round the site of inoculation, or may extend over the entire side, and even into the neck, and in some cases over the median line to the opposite side. The oedema almost invariably assumes a *gelatinous* appearance, in some cases composed of pure yellowish serum and in others of serum stained with blood. The subcutaneous lymph glands of the axillary, inguinal, and cervical regions are usually haemorrhagic and swollen, the swelling being greatest on the side of inoculation. The pleural cavity frequently contains a considerable quantity of straw-coloured clear fluid, sometimes amounting to 12 c.c. or more. The peritoneal cavity occasionally contains similar fluid, but less frequently and in smaller quantity.

Both the visceral and parietal layers of the peritoneum are usually injected and ecchymoses are present in many cases. The *suprarenal glands* always show intense congestion and in some instances may be haemorrhagic, the colour of the organs varying from pink to a very dark red.

These are the principal lesions encountered in experimental diphtheria in guinea-pigs. Other gross lesions are, however, frequently met with. The lungs often show areas of congestion, and even consolidation. The liver is generally congested and often fatty. It occasionally shows on its surface macroscopic lines and dots, varying in size from a pin's point to a pin's head. Though the spleen is not perceptibly enlarged in all cases, yet in some it is markedly enlarged, and the kidneys are frequently hyperaemic.

The mesenteric, retroperitoneal, mediastinal and bronchial glands are very frequently enlarged and often reddened, and the agminated glands of the coecum and ileum are generally abnormally prominent.

(*b*) *Histological Lesions.*

The seat of inoculation. Bacilli are to be found in the necrotic focus both free and in leucocytes, but they do not usually occur in the oedematous fluid at a distance from the focus. Sections made from the seat of inoculation show the bacilli in great numbers, and those stained by Weigert's fibrin method exhibit in a striking manner the bacilli and fibrin.

Welch and Flexner (1891) summarise the local changes in the following sentences. "The local action of the bacilli is of a most intense character. There is emigration and great destruction of leucocytes shown by the disintegration of their nuclei; the fixed cells of the part have undergone a similar fragmentation, nuclei of connective tissue and muscle have succumbed, and leucocytes have wandered into these areas, many of the latter being also destroyed."

Axillary and Inguinal and other Lymph Glands.

A very minute account of the histological changes found in the lymph glands is given by Welch and Flexner (1891) who regard the changes as very typical: "There are haemorrhages under the capsule and into the substance of the gland. The blood vessels here, as elsewhere in the body, contain a greatly increased number of leucocytes. The cells of the

glands are the seat of great changes. The principal lesions are in the lymph follicles, the lymph cords and lymph sinuses being also affected, though in less degree. Different follicles are affected in different degrees, but in no instances were the lesions entirely absent. The lesions consist of a marked alteration in the number, character, size, staining capacity and configuration of the nuclei making up the parts affected. The cell bodies are altered also, and an increased number of cells differing from the lymphoid type are found. The first thing that attracts attention is the unusual number of deeply staining bodies in the tissue. These bodies are observed to vary in size and shape, and under a sufficiently high power some of them are recognised as nuclear figures.

They are usually however globular, and under a magnifying power of four hundred, range from fine dust-like particles to larger particles appearing with this power the size of a pin's head. The finer particles are often aggregated into globular masses, which are now free and now enclosed in cells. There are, again, deeply staining particles present, which show decided bizarre forms. Imperfect crescents, flask-shaped, bladder-like, whetstone, angular, and dumb-bell forms are more or less common. Occasionally, nuclei appear as if one end were drawn out and constricted into a ball-like protuberance that is being pinched off. The globular particles are, at times, grouped together with the bizarre forms into larger masses; what particularly distinguishes these bodies from the normal nuclei which remain is the intensity with which they stain.

All the chromatin particles, as before mentioned, are not within cells; indeed, as a rule, they do not occur in cells, though the number within cells varies considerably. In some glands much of this material is contained within globular cells several times larger than the lymphoid cells. In some sections these cells are observed to be present in considerable numbers, partly devoid of stained particles, or nearly so, but usually they are full of nuclear detritus.

In certain spots there is almost an absence of stained cells and particles. In these places it is possible to distinguish outlines of cells, then a finer, granular, somewhat refractive, at times reticulated, material, and here and there a deeply stained particle. But there are always a few cells remaining that stain more or less, and amongst these cells are round cells, larger than the lymphoid cells. The portion stains with Weigert's fibrin stain in such a manner as to indicate that the granular material is largely made of fibrin or of a substance allied to fibrin. There occur in the lymph sinuses more especially a considerable number

of round, or slightly oblong, cells, quite colourless in appearance, containing red blood corpuscles."

"An alteration similar to that just described is found throughout the lymphatic structures of the body: in the spleen, mesenteric glands, retroperitoneal glands, intestinal lymphatic apparatus (Peyer's patches, solitary follicles, and diffused lymphatic tissue), the bronchial glands, mediastinal glands, and cervical glands. The only variation is one of degree. As far as our study has gone every gland examined has been more or less affected. The *Spleen* is often very rich in nuclear fragments and foci of coagulation necrosis."

Suprarenal Glands.

The blood vessels are congested and haemorrhages are frequently present. Occasionally the medullary cells are distinctly hyaline (Welch and Flexner, 1891).

Lungs.

There are often haemorrhages under the pleura, and all the blood vessels of the lungs, including the capillaries, are often distended with blood, and even haemorrhages into the alveoli may be found. An exudation of leucocytes and fibrin may occur into some of the alveoli. Fragmentation of the nuclei, except in the epithelial lining of the larger bronchi, is not common in the lungs (Welch and Flexner, 1891).

Liver.

In sections of the liver the dots and lines already mentioned are found to be areas of dead hyaline liver cells. Leucocytes are commonly found in such foci. These appearances were first observed independently by Welch and Flexner (1891) and Dubief and Bruhl (1891) about the same time. Welch and Flexner (1891) called attention to intralobular haemorrhages due to rupture of the walls of the central veins of certain lobules, and stated that the walls of these vessels, which were quite refractive in the fresh state, showed after hardening a characteristic hyaline appearance.

Kidneys.

Fatty changes are found in the epithelium of the tubules and glomeruli of the kidney. Welch and Flexner (1891) laid stress on the hyaline alteration of the glomerular capillaries and smaller arteries,

and observed that a hyaline substance completely filled the lumen of some capillaries.

Heart.

Fatty degeneration is commonly found in the heart muscle. According to Flexner (1894) this depends more on the intensity of the poison than on the time of its action. Mollard and Regnaud (1897) found various degeneration changes, such as alteration or disappearance of striation and hyaline changes, as well as interstitial sclerotic lesions in the heart muscle of guinea-pigs dying 17 days after inoculation.

Vessels.

Mollard and Regnaud (xi. 1897) occasionally met with endarteritis in chronic cases. The same authors (vii. 1897) once met with atheroma of the aorta in a guinea-pig dying eight months after the injection of dilute toxin.

Muscles.

Abbott and Ghriskey (1893) noticed in sections made through the seat of inoculation that many of the muscle fibres were in a condition of hyaline degeneration and were here and there invaded by diphtheria bacilli. Sometimes the penetration would occur through the side of the fibre, sometimes from one end (Pl. XIII, fig. 3).

Brain.

Degeneration of the cells of the brain has been noticed, especially in prolonged cases, by Berkley (1897) and others.

Intestines.

Welch and Flexner (1891) carefully studied the intestinal lesions. The cells of the villi and their epithelium showed lesions which varied in intensity. "The most striking changes consisted in a fragmentation of the nuclei of the cells in the villi, especially of those surrounding the central vessels, a disappearance of a large number of cells and the presence of large round cells, similar to those described in the part of the lymph glands most affected. These larger cells often showed a very slight staining power, and shadows of cells were not uncommon. The nuclei of the epithelium were distinctly and extensively fragmented. Nuclear figures were to be seen and saprophytic bacteria were found in the necrotic tissue. These fragmented nuclei partook

of the same characters as those already described, and exhibited the same intense affinity for staining agents."

Cells in the pleural effusion.

Courmont and Arloing (1901) found that the cells in the pleural effusion in most guinea-pigs consisted almost entirely of various forms of lymphocytes. In one animal only were polynuclear cells present to the extent of 25%.

The writer has recently made differential leucocyte counts in films made from the deposit obtained by centrifugalising pleuritic effusion, which do not bear out the above statement. In ten examples the proportion of the various cells in each case was nearly identical.

Hyaline cells and lymphocytes	...	...	44·99%.
Finely granular eosinophile cells	...	...	2·06%.
Coarsely granular eosinophile cells	...	...	52·93%.

Leucocytes.

The number of leucocytes in the blood is increased (Park, 1900).

Intraperitoneal inoculation.

Intraperitoneal inoculation produces similar changes to subcutaneous inoculation, except for the absence of subcutaneous lesions. At the autopsy the inflammatory manifestations in the peritoneum are more marked. Roux and Yersin (1889) were the first to point out that with intraperitoneal inoculations these animals take longer to succumb, than when subcutaneous injections are given.

PLATE XIII.

Fig. 1. Area of necrosis at the seat of inoculation in cow No. 1 (p. 285), showing the condition of fragmentation of the cell nuclei and clumps of irregularly stained *B. diphtheriae*. Stained with Bismarck brown and Gram's method. (In the original plate the bacilli appeared violet, the tissues brownish-yellow, the nuclei etc. darker brown.) Leitz $\frac{1}{12}$, Oc. 4. (From Abbott (1893), Plate, fig. 1.)

Fig. 2. Section through focus of leucocytes located in the omentum of a guinea-pig between the layers of the peritoneum. Leitz Obj. 3, Oc. 1.

Fig. 3. Hyaline muscle fibres from the seat of inoculation showing penetration of the bacilli into them. Stained with eosin and methylene blue. Leitz Obj. 7, Oc. 3.

Fig. 4. Contents of one of the foci (Fig. 2) dried upon a cover-slip and stained with Loeffler's blue. Leitz $\frac{1}{12}$, Oc. 3.

(Figs. 2, 3 and 4 are from Abbott and Ghriskey (1893), Plate, figs. 1, 4 and 2. Figs. 2 and 3 in the original are stained red and blue, and fig. 4 blue.)

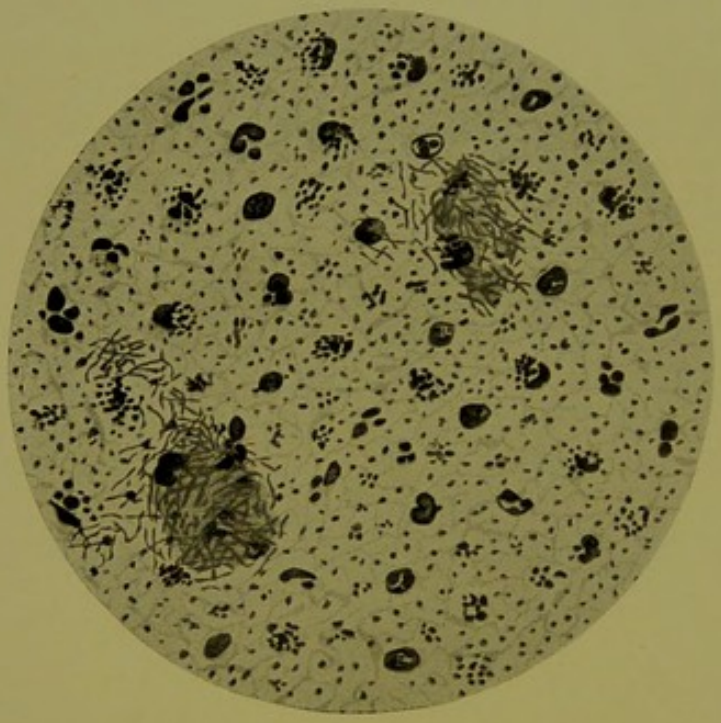


Fig. 1.

Fig. 2.

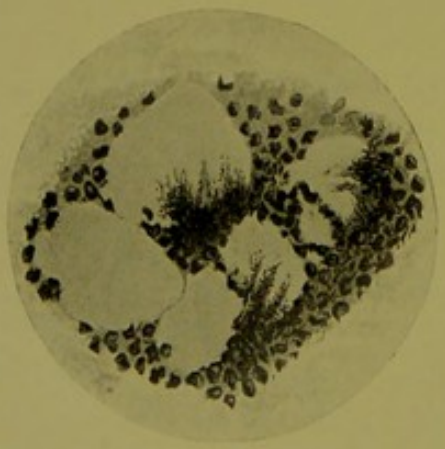


Fig. 3.

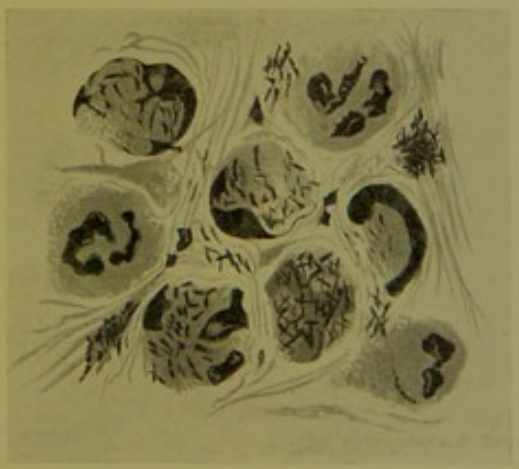


Fig. 4.

Tracheal inoculation.

By inoculating the abraded mucous membrane of the trachea with cultures a pseudo-membrane can frequently be produced. Roux and Martin (1894) observed the extension of such membranes down to the primary ramifications of the bronchi. Belfanti (1896) produced only bronchopneumonia by the injection of cultures into the uninjured trachea, but similar experiments by Henke (1898) gave negative results.

Inoculation of mucous surfaces.

Loeffler, Roux and Martin (1894) and many later observers have produced pseudo-membranes by the inoculation of the abraded mucous membrane of the vagina. Henke (1898) observed that sometimes oedema alone without membrane was produced. These pseudo-membranes resemble histologically those of the human subject.

Cornea.

Pseudo-membranous inflammation may be produced by rubbing cultures into the abraded surface of the cornea.

Inoculation into the testes.

Abbott and Ghriskey (1893) were the first to describe certain lesions of the omentum, which could be only produced with certainty by the injection of cultures into the testes. The lesions consist of minute yellowish, lens-shaped foci located in the omentum, usually between its peritoneal layers, and are most common near the free margin. When visible to the naked eye they are rarely larger than the eye of a small cambric needle and of about the same shape. The histological structure of these nodules is simple, the foci consisting of polynuclear leucocytes, the majority of which contain diphtheria bacilli (Pl. XIII, figs. 2, 4).

"A point of particular interest in connection with the cases in which the injection had been made into the testicle was the diminution of oedema of the superficial tissues, and its increase in the tissues of the abdominal cavity. In two of the cases straw-coloured fluid was not only found free in the peritoneal cavity, but the small intestine was practically filled with fluid and its walls were intensely oedematous."

Paralyses.

Various paralyses accompanied by lesions of the nerves and cells of the central nervous system may be produced in guinea-pigs and other animals by the injection of cultures simultaneously with or followed by antitoxin, or by the injection of small doses of toxin. The paralytic phenomena are dealt with later (Section V).

II. The Lesions of Experimental Diphtheria in Rabbits.

Guinea-pigs have been almost universally used to determine the identity and pathogenic powers of diphtheria bacilli. Rabbits are, however, susceptible to inoculations of diphtheria bacilli and their toxins, though life is more prolonged than in the case of guinea-pigs. These animals have, however, been used for the study of various lesions.

Time of Death.

Death may occur in four days with the injection of virulent cultures, or life may be prolonged for months when small doses of old cultures are given.

Leucocytes.

Schlesinger (1902) studied the blood cells in rabbits inoculated with virulent cultures. He observed that two hours after injection there was a hypoleucocytosis, but later a hyperleucocytosis.

Subcutaneous inoculation.

The animals die in four days or more, depending on the quantity injected. The following lesions are found at the autopsy: *subcutaneous oedema* is always present and is often very extensive, and sometimes it is haemorrhagic. The axillary and inguinal glands are swollen and the histological lesions are found to be the same as in guinea-pigs.

The *liver* is friable and yellowish with fatty degeneration (Roux and Yersin, 1888). In one instance Welch and Flexner (1891) found an extensive diffuse fatty degeneration of the liver tissue. The cells stained very imperfectly, many not at all. The nuclei of the liver cells were in many places in a state of disintegration or fragmentation; a few of division. The cells throughout entire lobules were affected.

The *lungs* are usually not affected, and exudation into the pleura is exceptional.

The *kidneys* generally show fatty changes in the epithelium of the tubes and glomeruli.

Fatty degeneration of the *heart* is very common. Mollard and Regnaud (1897) observed well-marked atheroma of the aorta in one instance in a rabbit which died six months after the injection of toxin.

There is usually much congestion of the *mesentery* and *omentum*, and ecchymoses are frequently present. The *intestinal villi* show the same changes as in guinea-pigs.

Trambusti (1896) investigated the *marrow*, and found that in the early stages there was an unusual amount of cell division, but as the disease progressed degenerative changes were found in the giant cells and leucocytes.

Crocq (1895) made careful investigations on the *nervous lesions* occurring in rabbits inoculated with four months' old cultures, under which conditions the animals lived for periods varying from one to three months. He states that lesions are found in the cord and nerves, rarely in the medulla, and never in the brain. These lesions consist of swelling of the nerve cells, loss of their protoplasmic prolongations, disappearance of nuclei, and extensive proliferation of the cells of the ependyma. Acute myelitis is rare. The lesions of the nerves appear from the fifth day onwards. The axis cylinder segments, the protoplasm becomes more abundant, the myelin becomes granular and is absorbed, and finally the axis cylinder disappears. Of the nerve roots the anterior alone are affected. In the rabbit it seems that the cord lesions precede those of the nerves.

Intravenous inoculations.

Loeffler observed that death did not invariably follow intravenous inoculations, but Roux and Yersin (1888) found that all the animals thus treated died in about 60 hours with a dose of 1 c.c. Metin (1898) and others have found that intravenous injection is usually fatal.

At the autopsy Roux and Yersin (1888) found general congestion of the abdominal organs, dilatation of the vessels, swelling of the glands, marked nephritis, and fatty degeneration of the liver, which had a yellow tinge. In animals which did not die so rapidly typical paralyses followed.

Meyer (1902) produced endocarditis of the injured valves by direct injection of diphtheria culture into the heart.

Tracheal inoculation.

Loeffler, Roux and Yersin (1888), Bardach (1895), Henke (1898), and others have found that inoculations with cultures onto the abraded tracheal mucous membrane after preliminary tracheotomy produce pseudo-membranes. The disease so produced is almost always fatal. The rabbits breathe with difficulty, and the membrane is apt to spread from the trachea to the fauces. Oedema of the tissues and enlargements of the glands of the neck are constantly present. Henke (1898) states that the pseudo-membrane consists of a fibrinous network, enclosing in its meshes leucocytes and cast-off epithelium. In the place of the normal epithelium a cloudy layer is seen representing it. The submucous layer is infiltrated with leucocytes and shows exudation and haemorrhage. Bacilli are found in the membrane but not in the submucous layer.

Flexner and Anderson (1898) made very extensive investigations on the introduction of cultures through tracheotomy wounds without further injury to the mucous membrane. Only small patches of congestion occurred in the trachea, but the lungs were often completely consolidated and presented a peculiar semi-gelatinous appearance. On section oedematous fluid escaped in small quantities. In several cases cultures from these lungs were negative, but others yielded diphtheria bacilli. In some instances, however, pneumonic processes in the lungs were not provoked.

Cultures made from the lungs of rabbits killed 1, $3\frac{1}{2}$, 6, 12, and 18 hours after injection showed bacilli, but cultures from animals killed 24 hours after inoculation remained sterile. Their examinations proved that the bacilli were rapidly taken up by the cells, but not by the polymorphonuclear leucocytes.

Lack (1899) was able to produce a pseudo-membranous inflammation of the trachea in a rabbit with a culture derived from a case of fibrinous rhinitis.

Stecksen (1900) was unable to produce pseudo-membranes with killed cultures.

Conjunctival inoculation.

Babes (1890) experimented with a number of rabbits and found that inoculation of the conjunctiva with cultures gave rise to thick exudates in 24 hours. Some of the animals died in eight to 15 days, and some developed paralysis and eventually died. The exudate

consisted of a fibrinous material containing fragments of cells and a few bacilli, and histologically resembled human diphtheria membrane. Several subsequent observers have confirmed the observation that pseudo-membranes may be produced by inoculations onto the injured cornea and conjunctival mucous membrane.

III. The Lesions of Experimental Diphtheria in Dogs.

Dogs are susceptible to the action of the diphtheria bacillus and its products, but they have not been very extensively used for experimental purposes. The principal lesions produced by the subcutaneous injection of cultures are the same as in rabbits, extensive oedema, fatty lesions of the heart, etc. Lesions of the nervous system seem to be very easily produced in these animals, both by the injection of toxins and of living cultures. Roux and Yersin (1889) record experiments on dogs in one of which a dog died stupid and paralysed three days after subcutaneous inoculation, and in another in which the animal died in the same condition four days after tracheal infection. No pseudo-membrane was found in the trachea at the autopsy.

IV. Experimental Diphtheria in Rats and Mice.

It has long been known that rats and mice are extremely resistant to diphtheria bacilli and their toxins. Unless enormous doses are injected the animals are not affected. Roux and Yersin (1888, p. 661), for example, inoculated a mouse subcutaneously with ·3 c.c. and another with 1 c.c. and two others with 2 c.c. of a filtrate, which was capable of killing a 350-grm. guinea-pig in a dose of ·2 c.c. None of the animals were affected in any way. A young rat was inoculated with 2 c.c., also without any ill effect. Cobbett (15. iv. 1899) made some experiments to test the relative resistance of rats and guinea-pigs to diphtheria toxin. He concluded that "the white or black rat of 100 grams weight is only relatively insusceptible to the action of the products of the diphtheria bacillus, and succumbs to the subcutaneous injection of filtered cultures, in quantities which are, weight for weight, from 1500 to 1800 times as great as those which suffice to kill guinea-pigs of 250 grms.[1]" The tissues are but little affected locally by the injections of large quantities of filtrate, and have not been observed to suffer necrosis. These results are not due to the presence of anti-toxin in the blood, as it has never been found in the blood of normal rats.

[1] Goodman (15. vi. 07) finds that it requires 3500 times the guinea-pig lethal dose to kill full-grown rats.

Roux and Yersin (1888) found that to kill a mouse it was necessary to inject 80 times the fatal dose for a guinea-pig, and that rats also resisted very large quantities.

Some of the writer's recent experiments with living cultures gave the following results. Out of five rats subcutaneously inoculated with large doses of virulent cultures, one died in four days with a collection of pus in the right pleural cavity and another between the liver and the diaphragm. Cultures from these abscesses yielded pure growths of the diphtheria bacillus. In another rat killed 120 hours after inoculation a small abscess containing diphtheria bacilli was found. The others were killed 48, 72, and 168 hours after injection respectively, but no lesions were discovered. From the first of these animals cultures were obtained from the site of injection, but not from the others. Rats inoculated with non-virulent diphtheria bacilli and Hofmann's bacilli showed no lesions. From some of these animals killed 48 hours after inoculation cultures could be obtained from the site of inoculation, but not from those killed later. The blood and organs in all cases were sterile.

v. Hibler (1896) calls attention to, and illustrates the presence of large numbers of diphtheria bacilli in the cells of the peritoneal exudate in mice five hours after the injection of diphtheria bacilli.

No instances of natural infection in guinea-pigs, rabbits, dogs, rats or mice have been recorded.

V. Experimental Diphtheria in Cats.

Klein (1888) produced diphtheritic lesions in cats by rubbing fresh diphtheria membrane onto the abraded surfaces of the cornea and palate. In 24 hours the parts became congested. After 48 hours the conjunctiva was greatly swollen, and the eye closed, and a muco-purulent material flowed from it. The cornea was opaque, and the palatine arch covered with a thin whitish-yellow film. After 72 hours the oedema and swelling had increased and the cornea was ulcerated and covered with an adherent membrane. The palate symptoms increased up to the 10th day and then gradually subsided. The eye symptoms only began to subside in two or three weeks. Eventually both lesions entirely healed. A second series of cats inoculated from the first went through the same course of events, with the exception of one which died on the 4th day. Similar lesions were produced in cats by the application of diphtheria cultures to the abraded surface of the conjunctiva (1889, p. 157).

Subcutaneous inoculation.

Klein (1889, p. 165) made several observations on the effects of subcutaneous inoculations of diphtheria cultures in cats. Six cats were inoculated in the groin and all showed considerable swellings on the 3rd day. Two died on the 5th day, two on the 10th day and two on the 11th day.

The autopsies in all cases showed extensive subcutaneous haemorrhagic oedema, and an almost gangrenous condition of the tissue in some places. The spleen and liver were only slightly congested, but the condition of the *kidneys* was remarkable. These organs were enlarged and the medulla congested, but the cortex was gray or yellowish-white in colour. Under the microscope the cells of the renal epithelium, especially those of the convoluted tubules, showed extensive fatty degeneration and breaking down into granular débris. Seven other cats similarly inoculated died between two and seven days after injection, and showed the same changes. Two cats inoculated simultaneously on the cornea and subcutaneously in the groin showed marked eye changes, with only slight signs on the groin. When killed after 19 days only slight renal changes were found.

Tracheal inoculations.

Broth cultures injected directly into the trachea by means of a syringe pushed through the anterior wall caused death in one animal in 26 hours, and another became paralysed in the hind limbs and very ill on the 6th day. The autopsy on the second animal showed a bronchopneumonic condition of the lungs, enlargement and fatty degeneration of the liver, and enlargement of the kidneys. The cortex of these organs was a uniform whitish-yellow mass. In microscopic sections of the lung characteristic diphtheritic false membranes were found in the bronchi and fibrinous exudation in the infundibula. Diphtheria bacilli were cultivated from the lungs (Klein, 1889, p. 159).

Welch and Abbott (1891) found that the application of cultures to the abraded tracheal mucous membrane in kittens produced typical diphtheritic false membranes in the trachea. The animals died in a few days.

Feeding experiments.

Klein (1890, p. 229) fed two cats on several occasions with milk mixed with diphtheria bacilli. The animals became thin, but showed no prominent symptoms. They were eventually killed after three and

four weeks respectively. Both showed greatly enlarged livers and large kidneys with a white cortex, and the lungs of one showed patches of gray hepatisation. Klein remarks that "from this experiment it is seen that by repeated feeding with cultures of the diphtheria bacillus distributed in milk, unquestionable diphtheria disease can be produced in the cat."

According to all Klein's experiments a fatty condition of the cortex of the kidney is very characteristic of diphtheria in cats.

Accidentally acquired diphtheria.

A cat placed in a cage with another, which had been inoculated on the cornea, also developed a conjunctivitis which was less severe, but resulted in ulceration (Klein, 1889, p. 158).

During some of Klein's (1889, p. 174) experiments on cows (p. 283) the cats in a certain shed were fed by mistake on milk containing diphtheria bacilli. In this shed a series of animals became ill during a period of two months, though healthy when procured. They suffered from an acute catarrhal affection of the conjunctiva and respiratory passages. Sixteen animals in all were affected and seven died after becoming much emaciated. At the autopsies the prominent pathological changes were lobular pneumonia, with fibrinous exudation containing diphtheria bacilli in the bronchi and infundibula, and enlarged kidneys with fatty degeneration of the cortex. In one animal there was a false membrane in the larynx and trachea in which diphtheria bacilli were found.

Nine months (1900, p. 235) later a similar outbreak occurred, but the source of infection was not found. In those animals which died the gross anatomical changes were similar to those just described, and in one there was a membrane in the larynx and trachea containing morphologically typical diphtheria bacilli.

The natural disease in cats.

A general impression prevails that cats contract diphtheria from human subjects, and very numerous instances are quoted in the literature[1] of cats, apparently suffering from diphtheria, communicating it

[1] Instances are given in considerable detail in the following papers:—Turner, G., *Local Government Board Report*, Vol. xvi. p. 309, 1886. Bruce-Low, *Ibid.* Vol. xviii. p. 131, 1888. Letters by Drs Downes, Shirley-Murphy, and Thersfield quoted by Klein, *Ibid.*, Vol. xix. p. 163, 1890. Symes, J. O. (6. vi. 1896), *Brit. Med. Journ.*, Vol. i. p. 1385, and Gray, H. (31. iii. 1896), *Journ. of Comp. Path. and Therap.* Vol. ix. p. 46.

to children, and other examples are cited of cats, although remaining quite well, being the only probable carriers of infection. Very few of these cases seem to have been bacteriologically examined, and in no instance has the presence of diphtheria bacilli been satisfactorily proved.

Klein (1889, p. 162) made autopsies on several occasions on cats which were regarded as carriers of the disease. One had become paralysed, and all showed the lesions which he described as characteristic of experimental diphtheria in cats. Diphtheria bacilli were not, however, cultivated from any of them.

Dowson (1895) records the examination of a cat which "was undoubtedly suffering from diphtheria." This animal became ill 14 days after a child in the house had died of diphtheria. It was killed on the 8th day of the disease. The autopsy showed the lungs to be in a condition of disseminated pneumonia, but all the other organs were healthy including the mouth, nares, larynx, and trachea. The kidneys were not examined. Cultures made from the lungs gave pure cultivations of an organism morphologically resembling the diphtheria bacillus, which was not, however, further investigated. In another cat, which he examined under similar conditions, the kidneys were found to be in the condition of fatty degeneration described by Klein, and the lungs showed some suppurating areas. From the latter, amongst other organisms, a few colonies of bacilli resembling those found in the first cat were discovered.

As an example of a case in which a cat was the probable carrier of the disease, an interesting observation by Low (1888, p. 131) may be quoted: "A little boy was taken ill with what turned out ultimately to be diphtheria. On the first day of his illness he was sick, and the cat which was in the room at the time licked the vomit off the floor. In a few days (the child meanwhile having died) the animal was noticed to be ill, and her sufferings were so severe and so similar to those of the dead boy that the owner had her destroyed. During the early part of its illness this cat had been let out at nights in the back yard as usual. A few days later the cat of a neighbour, who lived a few doors further off, was noticed to be ill. It had also been out in the back yard at night. This second animal, which, however, recovered, was the pet and playfellow of four little girls, who, grieved at the illness of their favourite, nursed it with great care. All four girls developed diphtheria, their mother being convinced they got it from the cat, and indeed no other known source of contact with infection could be discovered."

VI. Experimental Diphtheria in Cows.

Klein (1889, 1890) made several experiments on cows for the purpose of determining whether cows could be so infected with diphtheria bacilli that the latter made their appearance in the milk. The first experiments (1889, p. 168), simultaneously carried out on two cows, are quoted below at some length. Two healthy cows were chosen which had calved respectively three and four weeks previously. Each received under the skin of the left shoulder one hypodermic syringe full of broth culture of very virulent diphtheria bacilli incubated for three days at 37° C. On the third day a soft tender tumour about the size of an orange could be felt in each at the site of injection. The tumours increased in size for a few days, and then gradually decreased and became firm. The only constitutional disturbance noted during the first week was a slight rise of temperature during the 2nd and 3rd days in both cows.

On the 4th day a small vesicle appeared on the hind teat of cow No. 2, and others on the udder near to it. On the 5th day the vesicle had become covered with a brown crust, and the skin around was indurated. At this time there were on the udder several vesicles, some containing clear serum and others containing pus. All were slightly raised on a slightly injected corium. On the removal of one of the crusts an ulcer was exposed covered with a purulent film. The pustules and crusted ulcers differed in size; some measured not more than $\frac{1}{8}$ of an inch in diameter, others measured up to $\frac{1}{2}$ inch. The numbers of the vesicles had increased on the 6th day. In all cases there first appeared a vesicle filled with clear serum, which became a pustule, and later an ulcer covered with a crust, which gradually healed, the whole process occupying 6—8 days. From these lesions diphtheria bacilli were recovered in culture. A similar series of lesions occurred in cow No. 1.

Cow No. 1 became very ill by the 13th day and died on the 14th. At the autopsy a tumour about four inches square was found at the site of injection, consisting of laminae of fibrous tissue and necrotic material, and closely connected with the skin and underlying muscle. On section clear fluid oozed from the mass. The neighbouring lymph glands were enlarged, the upper lobes of the lungs and the kidneys congested, and the liver showed numerous gray necrotic patches. Cow No. 2 became ill and was killed on the 25th day. The lesions found at the autopsy were very similar.

'Microscopic sections made from the substance of the tumour showed necrotic reticulated masses containing large and small aggregations of the diphtheria bacilli. These aggregations of bacilli extended into the muscular tissue nearest to the septa, in fact the implication of the muscular fibres by the gradual growth into them of the masses of bacilli could be easily traced. Further, careful microscopic examination of the masses of bacilli in the necrotic portions of the tumour, as also of those implicating the muscular fibres, revealed the following remarkable fact, namely, that many bacilli had become threads, some of considerable length, and containing granules in their course, characterised by intermediate or terminal buds or swellings, spherical, oval, or flask-shaped. In a word, the organisms had taken on forms, which are but exaggerations of what has been before observed in cultures of this bacillus." "That these threads in the tissue of the tumour had been really developed from the diphtheria bacilli was proved by the circumstance that all intermediate forms between the typical diphtheria bacillus and these hyphae-like threads could be everywhere seen in the same microscopic section: and as well by the fact that every one of the colonies in the cultures obtained from the tumour were found to be pure diphtheria bacilli."

On the 5th day of the experiment a teat of cow No. 1, which was still normal, was cleansed and scrubbed, and milk collected from it into sterile tubes with every precaution against accidental contamination. From this milk diphtheria bacilli were cultivated in small numbers (about 32 colonies per c.c.).

With scrapings taken on the 6th day from the eruptions of cow No. 2 two calves were inoculated in subcutaneous incisions of the belly and groin. On the 6th day after the inoculation red papules appeared, and on the 8th vesicles formed, and continued to appear for 18 days, going through the stages already described in the cows. The animals developed a muco-purulent discharge from the nose, and a cough, and showed difficulty in breathing, and they fed very little. The lung trouble increased, and they became thin, and were killed on the 25th day. The autopsies showed enlarged oedematous inguinal glands, general congestion of the lungs with consolidation of the upper lobes, and well marked fatty degeneration of the cortex of the kidneys.

Further experiments with four cows (subcutaneous inoculation of cultures) resulted in the death of the animals, and similar lesions were found at the autopsies as in the first animals, but the teats and udders remained normal and no diphtheria bacilli could be cultivated from the milk.

Another set of similar experiments with two cows (Nos. 7 and 8) resulted, however, in the formation of eruptions on the teats and udders similar to those in cows 1 and 2. From the milk of cow 8 diphtheria bacilli were isolated. Inoculations with old, rather attenuated, cultures of diphtheria bacilli only produced slight swellings and little constitutional disturbance.

Two other cows (Nos. 9 and 10) were subcutaneously inoculated with broth cultures, and in one an ulcer appeared on the base of a teat on the 8th day. Each cow had a sucking calf, both of which on the 9th day showed vesicles surrounded by red areas on the upper lip. In the next few days several vesicles developed round the earlier ones. No cultures appear to have been made from these lesions. No diphtheria bacilli were found in the milk of these cows on the 10th day after inoculation, *i.e.*, on the day subsequent to the appearance of the eruption on the lips of their calves. A calf subcutaneously inoculated with a virulent culture developed a large, soft, tender swelling over which the epidermis desquamated in large flakes. Cultures of diphtheria bacilli were obtained from the swelling.

Cutaneous inoculations.

Five calves were inoculated along incisions in the abdominal wall, two with cultures of diphtheria bacilli, and three with fresh human diphtheria membrane. In three days the incisions became tumid and red, and later the central portion developed a thin linear crust with indications of vesiculation at the edges. In those inoculated with cultures the condition subsided, but in those infected by means of membrane the condition went on to ulceration.

Feeding experiments.

Feeding experiments with broth cultures on two cows produced no results.

Abbott (1894) repeated some of Klein's experiments on the subcutaneous inoculation of cows, but did not confirm his results. One of the two cows he used was tuberculous and died in 16 days. No eruptions occurred on the teats, but a tumour developed at the site of inoculation. The tumour consisted of a peripheral zone of fibrous tissue and an internal necrotic mass containing muscle fibres and connective tissue in various stages of degeneration. In some parts there was only a reticulum of fibrin with fragments of cells. Although

clumps of diphtheria bacilli could be seen in sections of the tumour none could be cultivated (Pl. XIII, fig. 1). The second cow was healthy and suckling a calf. She developed a swelling at the site of inoculation but no eruptions on the teats, and was killed on the 20th day. At the autopsy the lungs were found to be normal, but the liver showed three yellowish fatty patches and the kidney one small fatty area. The milk of both the animals was studied for nine days after the inoculation and all suspicious bacilli were isolated and tested, but no diphtheria bacilli were found. Abbott consequently thought that diphtheria bacilli did not pass into the milk as described by Klein. Ritter (1896) also attempted to repeat Klein's experiments, but failed to show that the diphtheria bacilli passed into the milk.

Klein (1894) replied to Abbott's criticisms, and raised certain objections to his experiments and deductions. He showed that Abbott had only seen the accounts of his first two experiments and not those of the remainder which confirmed them, and argued that the animals used by Abbott were not fit subjects for the experiment, in that one was tuberculous and the other in poor condition. He also considered that the cultures used were unsuitable, since they were not very virulent as proved by Abbott's experiments with them on guinea-pigs, the slight reaction produced in the cows, and his inability to grow them from the local lesion.

Spontaneous infection in the cow.

Thorne-Thorne (1891), in reviewing the chief epidemics of milk-diphtheria in England, sums up as follows: "On each occasion of milk-diphtheria to which I have referred there has been some evidence, more or less precise, of some cow ailment, so far trivial, it is true, as to be ignored by those versed in bovine diseases, but either affecting the physical properties of the milk, or being associated with some vesiculation and later on with 'chapping' or 'scabbing' of the udder and the teats."

Klein (1890) after his experiments pointed out that in two outbreaks of diphtheria, one near Croydon and the other near Bishop's Stortford, lesions occurred in the udders of the suspected cows, having the same characters as those produced by experimental inoculation. He did not claim, however, to have demonstrated bacteriologically either the diphtheritic character of the lesions or the presence of the bacillus in the milk. Although these facts were pointed out many

years ago, only two observations have been published claiming to have demonstrated the presence of diphtheria bacilli in the lesions of cows. One was the interesting outbreak investigated by Dean and Todd (1902). The milk of two cows was used chiefly by the family of the owner and his servants and the surplus was sold to a few individuals. Two of these persons suffered from typical diphtheria and several had sore throats. The cows were found to be suffering from a disease, which had commenced about 10 days before, characterised by the following lesions. On the udders and teats of both papules and ulcers covered with dark brown scabs were present. The papules were, on the average, the size of a pea, and had a markedly indurated base which extended into the subcutaneous tissue. No vesicles were seen at this examination. The majority of the lesions were in the form of ulcers covered with dry brown crusts and varied from 2 to 2·5 cms. in diameter. On removing the crust from one of these ulcers there was exposed a slightly moist, fairly smooth surface, with an elevated puckered cicatricial-looking margin. The largest lesion at this stage on the udder measured 2×1 inches.

In cow 1 there was no evidence of mammitis, there was an abundant secretion of apparently normal milk, and the general health appeared to be little, if at all, affected. In cow 2 there was a distinct mammitis affecting a posterior quarter of the udder. In this case the milk was scanty, ropy, and semi-purulent looking. Cultures, taken from the lesions and from the milk of both cows, showed typical virulent diphtheria bacilli, whose effects on guinea-pigs could be neutralised by antitoxin. The toxigenic power of bacilli from three sources, namely, from the lesions and milk of cow 1 and the throat of a patient, was tested, and was found to be the same.

As controls cultures made from the teats of 13 apparently healthy cows in a small dairy farm showed no diphtheria bacilli.

With a view of further investigating the condition a healthy cow (No. 3) was milked immediately after the diseased ones by the same attendant. This animal developed several vesicles on the teats, but diphtheria bacilli could not be grown from the contents. Crops of papules continued to develop for a fortnight.

Two calves were shaved and scarified on the abdomen and inoculated with crusts from the experimentally infected cow No. 3. These animals developed papules, followed by shallow ulcers covered with brown crusts, but diphtheria bacilli could not be found in the lesions.

From these experiments the authors came to the conclusion that

the lesions in the cows were not due to the diphtheria bacillus, but that the latter had probably been superadded to them in some way and multiplied in them.

The second example was investigated by Ashby (1906). By its distribution he became convinced that a diphtheria epidemic, which occurred between July 31st and August 26th, 1904, in the villages of Twyford and Ruscombe, containing about 2250 persons, was spread by infected milk. In all 75 persons living in 43 houses were attacked, of whom 64% were over 15 years of age. These cases were proved by bacteriological examination. Milk was supplied to the two villages by three dairymen, who may be designated X, Y, and Z. One of the houses invaded by diphtheria was supplied by X, 15 of them were supplied by Y alone, 17 were supplied by Z alone, and 10 were supplied by both Y and Z. The cows belonging to Y and Z were inspected on August 20th. It was found that all of the teats of two of Z's cows were badly ulcerated, and the teats of three other cows were affected to a lesser degree. Cultures taken from the ulcers on the teats of the worst two cases were examined at the Lister Institute of Preventive Medicine by Dr Alfred MacConkey, who isolated a diphtheria bacillus, fully virulent for guinea-pigs. Control guinea-pigs could be protected with antitoxin. From this organism a toxin was prepared of which $\frac{1}{40}$ c.c. killed a 245 grm. guinea-pig in 48 hours. Dr G. Dean also examined cultures independently, and proved the organism to be a true virulent diphtheria bacillus. Cultures from a sample of milk showed only non-pathogenic diphtheria-like bacilli.

"Whether the eruptive disease of the teat was a specific diphtheritic infection of the cow, or whether there was a specific contagious eruptive condition apart from diphtheritic infection cannot now be told; but it is certain that the diphtheria bacillus was present in the pathological lesions of the cow."

This report is accompanied by several excellent photographs of the teats of the affected cows as well as tables giving the results of the inoculation experiments on guinea-pigs.

Summary of Observation on Diphtheria in Cows.

Klein's experiments lead to the conclusion that cows can be experimentally infected with diphtheria, and that as a result of the infection certain lesions may be produced on the teats and udders which contain diphtheria bacilli, and that diphtheria bacilli may be present in the

milk apart from these lesions. The experiments of Abbott and of Ritter do not, however, confirm Klein's observations, and most of those who have criticised these experiments hold that there is no evidence that diphtheria is a bovine disease. Further experiments are therefore needed before a definite conclusion can be arrived at.

Several authors have pointed out that in epidemics of diphtheria, in which the milk has been strongly suspected as the agent by means of which the disease has been transmitted, some of the cows supplying the milk have been found to be suffering from lesions of the udder and teats. In only two cases, those investigated by Dean and Todd (1902), and Ashby (1906), has any bacteriological evidence of the infection of the milk by the cows been produced. In the former case, although the milk and the lesions on the teats contained virulent diphtheria bacilli, further experiments led to the conclusion that the diphtheria bacilli were not the causal agents of the bovine disease, but had been in some way superadded. In the latter case virulent diphtheria bacilli were found in the lesions on the teats, but neither their origin nor their relation to the ulcers was determined. On this subject also further careful investigations are needed[1].

VII. Diphtheria in the Horse.

The experimental results of the injection of living bacilli or toxins into horses are dealt with later (Section V). The only instance of a horse suffering from diphtheria and communicating the disease to man has been published by Cobbett (25. VIII. 1900). A little girl having fallen ill of diphtheria, Dr Fraser, the Medical Officer of Health of Portsmouth, while seeking the source of infection, found that a pony belonging to the child's father was ill with a purulent and slightly sanguineous discharge from the nose. Subsequently the animal suffered from enlargement of the glands under the tongue and tracheal obstruction, with difficulty of breathing and retraction of the abdominal wall. The bacillus isolated from the discharge was morphologically a short diphtheria bacillus, and on careful investigation was found to behave in all ways like the diphtheria bacillus. It formed a powerful toxin, and the effects of the injection of both living bacilli and of toxin were neutralised by antitoxin.

[1] For milk epidemics and diphtheria-like bacilli in milk see Index.

Cobbett considered this observation of considerable practical importance, since it proves that the horse is liable to nasal and laryngeal diphtheria, and points out a hitherto unsuspected channel by means of which the infection can be carried to man, and of scientific interest because it has a direct bearing on the question of the origin of antitoxin in untreated animals. He concludes by remarking that "the fact that diphtheria antitoxin is present in many horses in this country and on the Continents of Europe and America suggests that diphtheria is a common disease among these animals....It is therefore possible that the horse may be found to play a not inconsiderable part in the transmission of diphtheria."

A leading article in the *Journal of Comparative Pathology and Therapeutics* (29. IX. 1900, p. 248), while admitting that the above instance is one of equine diphtheria, severely criticises Cobbett's deductions as follows: "These, we venture to say, are very rash suggestions. Dr Cobbett is apparently under the impression that equine pathology is an unexplored field and that Veterinary Surgeons have been treating cases of equine diphtheria for generations without ever suspecting the nature of the complaint. In that he is certainly mistaken. The bacteriology of nasal discharges in the horse is probably as well known at this present moment as that of like conditions in man, and it is beyond doubt that diphtheria of the horse is an exceedingly rare condition. That it is maintained by transmission from animal to animal of the equine species is incredible, and it will probably be a long time before a second case of equine diphtheria is met with."

VIII. The Presence of Diphtheria Bacilli in the Blood and Organs of Experimental Animals.

While many of the earlier investigators were unable to detect any bacilli in the blood and organs, except in the neighbouring lymph glands, of animals dead from experimental diphtheria, later observers have been able to demonstrate their presence in some cases.

The bacilli can be detected microscopically, and also cultivated from the site of inoculation, in most animals unless death has been delayed for some days. The neighbouring lymph glands are also generally infected.

Roux and Yersin (1888) inoculated a series of guinea-pigs subcutaneously and killed specimens every two hours. After four hours oedema was found at the site of inoculation. The bacilli increased

in numbers up to eight hours, but after this time their numbers decreased. Except in one instance they were unable to find bacilli in the organs or pleural effusion. Métin (1898) observed a decrease in the numbers of bacilli two hours after inoculation, and Park (1900) says that the bacilli multiply but little in the tissues.

The largest number of experiments on this subject has been made by Wright (x. 1894). He examined the organs of guinea-pigs with the following results:

Organ	Number examined	Number of times diphtheria bacilli found	Percentage
Liver	155	19	12·2 %
Spleen	152	15	9·8 %
Heart's Blood	153	7	4·5 %
Kidney	151	4	2·6 %

Zarniko (1889) first observed the bacilli in the necrotic foci of the liver, and Abbott and Ghriskey (1893) in lesions of the omentum. Not infrequently the bacilli have been seen in sections and preparations from the organs, but cultures have remained sterile. Abbott (1894), for example, observed bacilli in the subcutaneous lesions of experimental cows, but was unable to cultivate them, and Flexner and Anderson (1898, Expt. H), although they saw bacilli in smears from the lungs, found that in some cases cultures remained sterile.

The latter observers remark "that even after considerable numbers of bacilli in pure cultures have been inserted through the trachea into the lung, their recovery from these situations was often attended with much difficulty and sometimes was impossible."

Métin (1898) found that in the case of rabbits, inoculated with pure cultures, the bacilli seemed to multiply in the organs after death. Cultures from the spleen immediately after death often remained sterile, but the bacilli could be obtained from it after incubation for 48 hours. He also endeavoured to ascertain when the bacilli disappeared from the blood after intravenous inoculation, by examining samples of blood drawn at intervals from the ear vein. A few colonies were obtained by culture half an hour after injection, but not four hours after. The animal was killed in six hours, but no bacilli could be found in the spleen before or after incubation. A second experiment gave the same results in regard to the presence of bacilli in the blood. This rabbit was killed 30 hours after injection. Although the organs and blood yielded no bacilli in cultures made immediately after death, colonies were obtained from the spleen after 48 hours' incubation. Animals killed 48 and 68 hours after inoculation gave

the same results. A series of experiments on guinea-pigs subcutaneously inoculated gave comparable results in regard to the rapid diminution of the bacilli at the site of inoculation and their presence in the spleen. Experiments, in which mixed cultures of diphtheria bacilli and cocci were introduced into the circulation of rabbits and under the skin of guinea-pigs, showed that the bacilli and cocci could be detected in the blood of the rabbits and in the subcutaneous oedema of guinea-pigs for many hours. Cultures from the organs and blood immediately after death also showed both organisms. Métin therefore thought that under these conditions the diphtheria bacilli were able to multiply in these situations during life.

IX. Diphtheria-like Organisms found in Animals[1].

Rats and mice.

Klein (1903) obtained from the hepatised lung of a white rat a bacillus closely resembling the diphtheria bacillus in its morphology, behaviour towards Neisser's stain, and cultural characters, which he has called *Bacterium muris*. It forms acid in glucose broth and is pathogenic to rats and guinea-pigs. In the case of an inoculated rat the whole of one lung became hepatised, and the other contained gray patches, and both showed ecchymoses. The liver and spleen were hyperaemic and the glands injected. In smears from the lungs large and small forms of the bacilli showing metachromatic staining were plentiful. Pure cultures were obtained from the lung and heart's blood. Another rat dying in 18 days showed similar changes. In guinea-pigs a hard tumour is formed at the site of injection which reaches a considerable size, and which gives rise to an abscess containing thick pus, in which the bacilli can be demonstrated. In all cases the animal recovers. Antitoxin has no effect.

Bergey (1904) found organisms, morphologically resembling diphtheria bacilli, in abscesses occurring spontaneously in laboratory mice, and Dean (1905, p. 103), while investigating a leprosy-like disease in rats, succeeded in obtaining from two of the affected animals cultivations of a diphtheroid bacillus. In young cultures on agar or serum "it has the form of a small diplococcus, or is sometimes very like Hofmann's bacillus. On the second or third day it becomes longer and has all the appearances of a typical diphtheria bacillus

[1] For an account of the diphtheroid bacilli found in milk see Chapter VIII.

with segmentation, club-shaped ends, etc., and later it may form short much segmented and branching filaments." On serum the colonies are indistinguishable from those of the diphtheria bacillus. On agar the growth is slower and the colonies more delicate. On broth it forms no surface film, but grows in the form of clumps along the test-tube wall, or as a flocculent deposit at the bottom. Glucose broth rapidly becomes acid. It forms no visible growth on potato. "This diphtheroid bacillus has a feeble pathogenic action on young rats. Three out of four young rats that received 2 c.c. of a broth culture a week old died within a week. Old rats proved resistant."

Horses.

Paffenholz (1895) found diphtheria-like bacilli in a tumour of the mamma in a mare.

Dogs and guinea-pigs.

As the xerosis bacillus appears to be a common inhabitant of the normal human conjunctiva, the writer (Graham-Smith, 1904, p. 307) made cultures from the eyes of a few animals to ascertain whether similar organisms are to be found there.

The eyes of three dogs, three rabbits, and 17 guinea-pigs were examined, and organisms closely resembling the xerosis bacillus were found in the eyes of the dogs and guinea-pigs, but not in the eyes of the rabbits.

Bacillus xerosis canis (Pl. XVI, fig. 14).

Origin. From the conjunctival sacs of the three dogs examined.

On serum the colonies only make their appearance after 2—3 days' growth, though in later subcultures the growth is a little more rapid. After 4—5 days' growth the colonies are of large size. Except for their size the colonies are indistinguishable in appearance from those of the diphtheria bacillus, but tend to adhere to the medium. The organisms are long, curved, and stain well, showing well differentiated, short, dark segments, separated by narrow light bands crossing the bacillus transversely. Clubbing in some specimens is well marked. These organisms resemble closely the pseudo-diphtheria type of Hofmann's bacillus (Pl. XV, fig. 7). By Neisser's method variously shaped polar bodies are seen in large numbers in each bacillus. Some are large and round, others elongated transversely across the bacillus, and many

are exceedingly small. The organisms are non-motile, and stain well by Gram's method. On agar after 2—3 days' growth small grayish colonies with irregular edges and darker centres appear. On agar stab cultures the surface growth is small and almost transparent, but in the depth a few medium-sized round colonies develop. On gelatin no growth was obtained. No visible growth occurs in potato.

Broth after 48 hours remains clear, but small granules are found at the bottom of the tube, which when shaken up float in lines as if held in position by invisible threads. The growth in glucose broth is similar, and its reaction after 48 hours is neutral. It is non-pathogenic to guinea-pigs.

This organism differs only slightly from the diphtheria bacillus in morphology, but differs from it in its growth on serum, gelatin and broth, and its reaction in glucose broth. It resembles closely in many respects the xerosis bacillus from the human eye.

Fourteen (82%) out of 17 guinea-pigs' eyes examined showed similar organisms. They resemble the organism just described in morphology, staining characteristics, and in their growth on all media except serum. On serum their colonies are the same in most cultures, but in some cases larger colonies develop which have a raised centre and raised rim.

These observations indicate that organisms closely resembling the diphtheria bacillus in morphology, and in many respects in cultural peculiarities, but totally unconnected with them, are common inhabitants of the conjunctival sacs of some animals.

X. The Lesions of Experimental Diphtheria in Birds.

Fowls, pigeons, and other birds may be killed by the inoculation of diphtheria bacilli, but except in the case of tracheal inoculations do not show very characteristic lesions.

Subcutaneous and intramuscular injections of doses of 1 c.c. of virulent broth cultures kill pigeons in 60 hours. In the former case a small layer of gelatinous oedema is observed and a general congestion of the internal organs without special lesions. Intramuscular injections produce the same general conditions, and the muscle is swollen, soft, yellowish in colour, and easily broken down. Bacilli can be isolated from the broken-down muscular tissue, and histologically the organisms may sometimes be seen penetrating the fibres. There is no marked congestion of the suprarenal glands.

Tracheal inoculations.

The majority of observers, who have made experiments on this subject, state that more or less extensive pseudo-membranes may at times be produced by inoculating the abraded mucous membrane of the trachea in birds. These lesions cannot, however, be produced with certainty. According to Henke (1898) only 25% of the inoculated birds exhibit membrane formation. While some observers have confirmed this observation others seem to have been unable to infect fowls in this manner. Harrison (1902), for example, could not produce any effects in fowls either by subcutaneous or submucous inoculations, or by rubbing cultures on to the abraded surface of the trachea.

XI. The Relationship of Avian to Human Diphtheria.

Diphtheria of fowls and pigeons has been made the subject of numerous investigations; and when we examine the literature, we are immediately struck with the differences of opinion regarding the disease. On one side we have those who believe that the one disease in man and in birds is identical; and, on the other side, those who believe that the one disease has no relation to the other.

The various writers and investigators on this subject may be conveniently grouped under two heads:

(1) Those who have investigated the disease as it occurs in fowls and pigeons, by the usual methods employed in working out infectious diseases.

(2) Those who have made observations without experimental research, and who did not employ bacteriological methods to support or controvert their views, either for or against the identity of the disease as it appears in birds and man.

(i) *Experimental Investigations on Avian Diphtheria.*

(a) Observations in which the disease was found to be due to organisms bearing no resemblance to the diphtheria bacillus.

Only a few of the more prominent investigations are quoted in which diphtheria-like organisms have not been discovered.

(1) *Bacilli.* Loeffler (1884, p. 482), isolated from the pseudo-membranes in the mouths of pigeons, which died from an infectious form of diphtheria prevalent in Germany, a bacillus, which, when

inoculated in pure culture, reproduced the disease. It was non-pathogenic to guinea-pigs, rats and dogs. Loeffler's observations were soon afterwards confirmed by Pütz (1887), Cornil and Babes (1890), Méguin (1891) and Ménard (1890).

Moore (1895) isolated a bacillus resembling that causing swine plague. Loir and Ducloux (1894, p. 599) studied in Tunis an outbreak which affected fowls, ducks, sparrows, pigeons and turkeys. From the tissues and fluids of all these cases they isolated a motile, non-liquefying bacillus with rounded ends which gave yellow colonies on potato. It did not stain by Gram's method, and was pathogenic for fowls, pigeons, ducks, sparrows and rabbits, but not for guinea-pigs. Cases of so-called diphtheria were common amongst those who attended the birds, and from one of these the same bacillus was isolated. The inoculation of the bacillus into healthy fowls gave them the disease.

Nocard and Leclainche (1903, *Les Maladies microbiennes des Animaux*, 3rd edition) state that the bacillus of avian diphtheria is analogous in its form to that of the haemorrhagic septicaemias.

Guérin (1903) has recently investigated several outbreaks of fowl diphtheria and isolated a cocco-bacillus which in pure cultures reproduces the disease in fowls. By successive inoculations on the conjunctiva of pigeons its virulence can be so raised that it produces death in fowls in 24 hours. He further succeeded in immunising fowls against the disease by means of a specific anti-serum. In consequence of his researches he came to the conclusion that human diphtheria is entirely distinct from that of the fowl. During three years this author never saw or heard of a case of human infection.

(2) *Cocci.* Cocci have been found in the false membranes by numerous observers.

(3) *Protozoa.* Pfeiffer (1889, p. 363), Babes and Puscariu (1890, p. 376), and Piana and Galli-Valerio (1894, *Moderno Zooiatro*, quoted by Harrison), as well as other later observers, have found flagellata and other protozoa in the throats of affected birds. In some cases they have been associated with Loeffler's bacillus of avian diphtheria.

Harrison (1902) has given the subject of avian diphtheria great attention, his work extending over a period of four years, and involving observations and inoculations on 300 fowls. He never met with the diphtheria bacillus in cultures from over 200 fowls, which had died from avian diphtheria or had been examined at various stages of the disease. His observations on the structure of the false membranes of fowls show that they are composed almost entirely of pus, some granular masses, and

débris of epithelial cells, and therefore differ entirely in their histological structure from the pseudo-membranes of true human diphtheria. He isolated specific bacteria from the diseased birds, and reproduced the typical disease in healthy birds by injection. The virulence of the organisms could be exalted by successive passages through pigeons. He also isolated from birds suffering from roup the *Bacillus pyocyaneus*, and reproduced the disease by its inoculation. Finally his experiments with the diphtheria bacillus convinced him that it was not pathogenic for hens.

(*b*) *Observations in which diphtheria-like organisms have been discovered and looked upon as the causal agents.*

De Verey (1895) stated that he found diphtheria bacilli in two cocks suffering from avian diphtheria, and regarded the bacilli as the cause of the disease, and Fagnet (1898) also asserted that he had found typical virulent diphtheria bacilli in one type of avian diphtheria. Gallez (1898) discovered in the nasal mucus of fowls, affected with contagious catarrh, a bacillus identical with the diphtheria bacillus in staining reactions and in cultural characters. Its virulence for guinea-pigs was very slight, though these animals could be killed with very large doses. Cultures inoculated into fowls reproduced the typical catarrh. Ferré (1898) describes a bacillus isolated from diphtheritic birds, which retains Gram's stain, and occurs in long and short banded forms. False membranes are produced in rabbits, pigeons and fowls by the inoculation of cultures, and paralyses in guinea-pigs and fowls by the injection of its toxins. He seems to have found the same organism in the throats and cloacae of healthy fowls. Gratia and Lienaux (1898) isolated from pigeons suffering from a diphtheritic disease an organism resembling the diphtheria bacillus in most of its characters. It was segmented, retained Gram's stain, showed polar granules, and varied greatly in length. In culture it grew well on serum, and produced a granular deposit and acid reaction in broth. No toxin was however produced. Gordon Sharp (1900) found a diphtheria-like bacillus in fowls suffering from roup, and Turner (1900) made similar observations. Neither of these authors seems to have tested the pathogenic properties of their bacilli.

Stevenson (1898), apparently on the results of experiments on diseased fowls with diphtheria antitoxin, stated that fowl diphtheria or "roup" was caused by the diphtheria bacillus and was identical with the human disease. Harrison (1902), however, showed that in his

cases neither anti-diphtheritic, nor normal horse, serum had any effect on the course of the disease. All the authors cited above consider that in some outbreaks at least avian diphtheria is caused by the true human diphtheria bacillus, though the organism is frequently of low virulence.

(c) *Observations in which diphtheria-like organisms have been discovered in diseased or healthy birds, but were not regarded either as the causal agents or as true diphtheria bacilli.*

McFadyean and Hewlett (1900) isolated and cultivated bacilli, morphologically resembling diphtheria bacilli, both from healthy pigeons and others suffering from pigeon "canker." They describe these organisms as resembling diphtheria bacilli in size and parallel arrangement. They retain Gram's stain, and show polar bodies by Neisser's method. Growth on serum varies, some examples producing dry and abundant growth like the xerosis bacillus, and others moist colonies like the diphtheria bacillus. These organisms produce acid and indol in broth, and are entirely non-pathogenic to mice and guinea-pigs, and in culture have no effect on pigeons. Harrison (1901) found the same organism in the throats of healthy pigeons. Guérin (1903) in 78 cultures from the throats of fowls twice isolated a bacillus morphologically resembling the diphtheria bacillus, but which produced no toxin, and he also states that Malvoz had once met with this organism. Streit (1904) also found similar bacilli which did not retain Gram's stain. The writer (Graham-Smith, 1904, p. 314) also found an organism resembling the diphtheria bacillus in the throat of a healthy fowl, and suggested the name *Bacillus diphtheroides gallinarum*. Its morphological and cultural characters are as follows:

Bacillus diphtheroides gallinarum (Pl. XVI, fig. 5).

Origin. From the throat of a fowl. There was a hard tumour on the side of the left mandible, but the bird was otherwise normal.

On *serum* the colonies after 24 hours' growth resemble those of the diphtheria bacillus. Later the margins become crenated. In the first cultures the organisms were long, curved, and clubbed, with three or four well-marked polar bodies. Slight swellings were present round the polar bodies. The rest of the protoplasm stained lightly, but slight signs of segmentation were present. They resembled closely the diphtheria bacillus shown on Pl. VI, fig. 1. In subcultures well-marked segments are seen. These organisms are non-motile, and retain the stain deeply by Gram's method. On *agar* small, filmy,

transparent, gray colonies are formed. The organisms are very long, thick, curved, clubbed, and well segmented, but no polar bodies are present. On agar stab cultures an almost transparent film is formed on the surface, and minute round colonies along the needle track. On *gelatin* after five days' growth minute, round, almost transparent colonies appear. On gelatin stab cultures there is very little surface growth, and a very scanty growth of minute colonies along the needle track. No visible growth occurs on *potato*. *Broth* remains clear and there is a slight granular deposit. *Glucose broth* shows a neutral, or slightly alkaline reaction. *Indol* is produced. Non-pathogenic to guinea-pigs.

As will be seen from the above description this organism very closely resembles the diphtheria bacillus except in its lack of virulence to guinea-pigs and in the production of an alkaline reaction in glucose broth.

Turkeys.

In studying an infectious disease of turkeys, characterised by gelatinous infiltration of the infra-orbital dilatation of the nasopharyngeal system of air-sacs, the writer (Graham-Smith, 1907) has encountered two species of diphtheroid bacilli, neither of which seems to bear any relation to the disease. The species which more closely resembles the diphtheria bacillus was found in groups usually composed of three to ten individuals, but occasionally containing as many as 40—50, in smear preparations of the gelatinous material. Most of the organisms were curved, showed well-marked segmentation, and well-defined polar bodies. In culture this species has the following characteristics.

On *serum* after 24 hours' growth at 37° C. the colonies are small, round, yellowish and dry-looking. After three days' incubation the colonies become very large. The centre is much raised and deep yellow in colour and is surrounded by a flat pale yellow zone. The whole colony has a very granular appearance. If the colonies are crowded together they coalesce to some extent to form a film which appears to have a wrinkled surface owing to the irregularities in the height of the component colonies. The bacilli bear a remarkable resemblance to true diphtheria bacilli. They are of medium length, with rounded ends, and are slightly curved. Clubbed extremities are common and many have irregularities in their length. Some branched forms were seen. They retain Gram's stain, show polar bodies, both terminal and central, with Neisser's stain, and differential staining of the protoplasm with methylene blue. In some dark and light bands alternate causing a segmented appearance, whilst in others these areas are irregularly placed. They are non-motile, do not form spores, and have no characteristic arrangement. On *agar* large

white granular, irregular, dry-looking, heaped up colonies are formed, which after a few weeks' growth increase in size and become wrinkled. The bacilli are shorter than when grown on serum and many coccus-like forms can be found after 48 hours' growth. On *potato* large, yellow, dry-looking, discrete colonies are produced. At first the bacilli are like those from the serum cultures, but later large irregular involution forms are common. Good growth occurs on *gelatin*, and large dull-yellow, granular colonies are formed. The medium is not liquefied. In *broth* a whitish wrinkled film is produced on the surface, and a yellowish flocculent deposit. Acid is produced in media containing *glucose*, *galactose*, and *laevulose*, but not in media containing *lactose* or *glycerine*. The organism is non-pathogenic to guinea-pigs.

(ii) *Opinions as to the Relationship of Human and Avian Diphtheria based on Clinical Observations.*

Clinical observations, however trustworthy, of the transference of a disease characterised by the formation of false membranes from birds to man or from man to birds, so long as they are unsupported by bacteriological proof, cannot be taken as evidence of the transmission of true diphtheria. Loir and Ducloux's (1894) observations clearly demonstrate this point. On this account, although there are many instances quoted in the literature of such outbreaks, only a few of the more noteworthy examples are cited.

Gerhardt (1883) published the report of an outbreak of diphtheria amongst two-thirds of the employees of a poultry establishment at Nesselhausen, where thousands of fowls had succumbed to diphtheria. One of the employees, pecked by a diphtheritic rooster on the hand and foot, subsequently showed false membranes in these situations. No cases of diphtheria were present in the environs of Nesselhausen at the time.

According to Paulinis (1888) the Greek island of Skiatos, in which diphtheria had never previously been known, was infected after the introduction of diphtheritic turkeys. Barbier (1899, p. 37) frequently saw diphtheria amongst fowls which lived beside isolated buildings for diphtheria patients, and records the case of a woman, 67 years of age, who was attacked by diphtheria after supervising the disinfection of a poultry building in which fowls suffering from diphtheria had been kept. No other case existed in the neighbourhood, and she had never been out of the house for the previous three weeks.

Debrie (1892) reported an interesting case. Some soldiers suffering from diphtheria were admitted to the hospital at Sebdou. Shortly

afterwards the fowls, which were looked after by a hospital attendant, showed symptoms of diphtheria.

On the other hand numerous observers have stated that they have never seen cases of human diphtheria amongst those who were in charge of diphtheritic birds.

Conclusions.

Those who have adopted experimental methods in investigating avian diphtheria have shown that the great majority of epidemics have been due to organisms totally distinct from the diphtheria bacillus. In a few instances only have diphtheria-like organisms been found, and most of these have been completely devoid of virulence for guinea-pigs. Moreover the results of experimental inoculation of the trachea in birds with diphtheria bacilli show that they are frequently resistant to this organism, and therefore unlikely to acquire the disease in epidemic form. The clinical observations, which support the identity of the two diseases, are not very convincing, owing to the lack of corroborative bacteriological evidence.

On the whole therefore these investigations point to the conclusion that true diphtheria, produced by the diphtheria bacillus, if it ever occurs, is a very rare disease in birds.

Friedberger and Fröhner (1904, p. 221) in their well-known work on veterinary pathology summarise the whole question of animal diphtheria in the following words:—"The diphtheritic diseases of domesticated animals are in no way related to human diphtheria. No indisputable case of the transmission of diphtheria to man from an animal has yet been proved. The cases recorded in the medical literature, of the alleged transmission of infection, especially those of chicken-diphtheritis supposed to have been conveyed to man, are, on closer examination, reduced to mere assumptions, the forming of which have been due to entire ignorance of veterinary pathology. Even the statement of Gerhardt—which is the only one of the kind worthy of being quoted—that in a chicken-hatching establishment two-thirds of all the workmen attending on the fowls which were suffering from diphtheritis, became affected with pharyngeal diphtheria, cannot be substantiated; for Gerhardt did not observe the case personally. Considering the wide distribution of chicken diphtheritis, especially in hatching establishments, and presuming that this disease is identical with human diphtheria, a very large number of people would necessarily become infected daily, and reports of such cases would not be, as

they are, extremely rare. We have examined thousands of domestic fowls and pigeons suffering from diphtheritis without having either seen or heard of a single case of infection. On the other hand, no indisputable proof has yet been given that any disease similar to human diphtheria has been transmitted, either experimentally or accidentally, to any of the domesticated animals."

Holmes (1904) in an article entitled "An Outbreak of Diphtheria associated with a similar Disease among Fowls and a Vesicular Eruption on the Udders of Cows," describes a very remarkable epidemic. Owing to its peculiar features it has not been included in the discussion on avian diphtheria, but it is recorded by itself. The outbreak occurred at Muktesar, a bacteriological station in the Himalayas, situated 23 and 16 miles respectively from the nearest European settlements, and 3 to 4 miles from the nearest native villages. The only inhabitants consisted of the staff and their families (four children). In June one child developed a nasal discharge, and a few days later another suffered from a disease which was diagnosed as diphtheria, and eventually died. Antitoxin was injected in this case, but somewhat late in the disease. There had been no case of diphtheria in the surrounding districts or stations for years, and no clue was obtained as to how the infection was brought. Two months previously a cow had been purchased which developed a cough and general illness, but recovered. Later a second cow was bought and placed near the other, and developed a similar illness. It was at this time that the child developed rhinitis and this circumstance led to a careful examination of the cows. It was then found that there were newly formed vesicles, ulcers, and pustules on the udder of the second cow, and healed lesions on that of the first cow. Cultures obtained from the vesicles showed bacilli somewhat resembling diphtheria bacilli, but non-pathogenic to rabbits and fowls. No further investigation of these organisms seems to have been made.

A month before the children's illness began a disease broke out amongst the fowls, which continued intermittently for several months, each outbreak following a turning up of the subsoil to a depth of 2 to 6 feet. From the soil on many occasions a bacillus was obtained identical in morphology with those derived from the birds, but non-virulent to rabbits.

Holmes carried out a large series of inoculation experiments with cultures and other material derived from the diseased children and fowls, which are summarised below:

A. Experiments on Animals with Cultures derived from the Sick Children.

I. *Fowls.* (1) A fowl inoculated on the abraded surface of the throat with a culture derived from the dead child died in seven days, with the larynx, trachea, pharynx, and nasal cavities covered with a fibrinous adherent membrane. No other lesions were discovered at the autopsy. Smears made from the membrane and heart's blood showed a bacillus with rounded ends and darkly stained polar granules. In cultures the organisms resembled diphtheria bacilli. (2) A fowl

injected subcutaneously died in 2½ days. At the site of injection a white fibrinous deposit was found between the muscles, and the surrounding tissues were injected and oedematous. Smears and cultures showed the same organisms as were found in the first fowl. (3) Intramuscular injection gave the same results.

II. *Bulls.* (1) A bull subcutaneously inoculated behind the shoulder with 5 c.c. of a 48 hours' broth culture developed very extensive oedema, extending down the leg, and died in 60 hours. At the autopsy an extensive yellow gelatinous oedema was found under the skin, and layers of white coloured fibrinous material between the muscles. The neighbouring glands were enlarged, congested, and oedematous, but no other lesions were found. A second bull inoculated in the same way died in 60 hours.

Six other bulls were also injected with minute doses.

Bull No. 3 received 1 loopful of broth culture and died in 5½ days.

,,	4	,,	1	,,	,,	,,	,,	6	,,	
,,	5	,,	1	,,	,,	,,	,,	6	,,	(culture 1 month old).
,,	6	,,	2	,,	,,	,,	,,	3	,,	
,,	7	,,	2	,,	,,	,,	,,	2	,,	(culture 6 weeks old).
,,	8	,,	2	,,	,,	,,	,,	64 hours		,, ,, ,,

In the oedema and heart's blood in all cases bacilli with bipolar staining, like those described in the fowls, were found, and in all those instances in which cultures were made from the heart's blood the organisms were isolated.

III. *Rabbits.* The pathogenic properties of cultures of these organisms towards rabbits were tested on several animals. All the rabbits died except one which had received a dose of antitoxin, and the bacilli were cultivated from the oedema and heart's blood.

	Method of inoculation	Dose	Result
Rabbit 1.	On abraded conjunctiva	?	Died in 4 days with fibrinous exudation on the conjunctiva.
,, 2.	On abraded surface of throat	?	Died in 36 hours with pseudo-membrane in trachea.
,, 3.	Subcutaneous	1 loopful	Died in 2 days. Yellow gelatinous oedema and injection at site of inoculation. Suprarenals reddish.
,, 4.	Subcutaneous	1 ,,	Died in 48 hours.
,, 5.	Subcutaneous	1 ,,	Died in 36 hours. Yellow gelatinous oedema and injection at site of inoculation. Suprarenals reddish.
,, 6.	On abraded surface of throat	?	Died in 5 days.
,, 7.	Culture applied to abraded throat the day after the injection of ·5 c.c. of diphtheria antitoxin	?	Remained well.

IV. A *cat* inoculated on the abraded surface of the throat remained well. Three weeks later it received a subcutaneous inoculation of one loopful and died in four days. A white fibrinous deposit was found at the site of inoculation,

surrounded by yellow oedema. Cultures were obtained from the blood, organs, and oedema.

V. A *dog* subcutaneously inoculated with half a loopful of culture died in three days. The subcutaneous tissue was deeply injected, and round the seat of inoculation there was a large amount of oedema.

VI. Two *rats* were inoculated subcutaneously each with half a loopful of culture, one died on the 3rd day and one on the 4th. There was oedema at the site of inoculation, and the organisms, recovered from the heart's blood, were fatal to rabbits.

The Natural Disease in Fowls.

A *fowl* (A) naturally infected showed fibrinous adherent membrane in the pharynx, larynx, trachea, and nasal cavities, but no other lesions. In smears from the membrane and heart's blood bipolar staining organisms, like those previously described, were found and isolated by culture.

Thirteen fowls and four ducks all showed more or less extensive lesions of this nature, and in two cases the oviducts of two diseased hens showed similar membranes. The bacilli seen in smears made from these membranes are described as being bipolar with rounded ends analogous to those of haemorrhagic septicaemia, non-motile, and not retaining the stain by Gram's method. In cultures they varied in form, often greatly resembling diphtheria bacilli. "In a blood-serum culture 24 hours old a thin transparent coating is visible, at the borders of which were isolated colonies of grayish colour and margin finely indented."

B. Experiments on Animals with Cultures and Material derived from Diseased Fowls.

I. *Fowls.* Several healthy fowls were inoculated with material from the false membrane by scraping the throat, and also subcutaneously with blood taken from the hearts of fowls which had died of avian diphtheria. Also many fowls and pigeons were inoculated with cultures from the blood and pseudo-membranes of diseased fowls, but except in one instance with no result. In order to raise the virulence of the contagion the method practised by Guérin was tried, namely, inoculating pigeons in the conjunctiva with some of the false membrane taken from an affected fowl. This also failed to have any effect. The one bird which was infected developed the same lesions as the birds which acquired the disease naturally.

II. *Bulls.* (1) A bull, which received subcutaneously five loops of a 48 hours' agar culture obtained from a diseased fowl (A), died in four days, with very marked oedema. The organisms were found in the oedema and heart's blood. (2) A second bull, which received 1 c.c. of oedematous fluid obtained from bull No. 1, died in four days. The autopsy showed extensive oedema, 1000 c.c. of yellow fluid in the pleura and 1500 c.c. in the peritoneum. The apex of the heart was covered with gelatinous oedema.

III. *Rabbits.* (1) A subcutaneous injection of 2 c.c. of broth emulsion of the pseudo-membrane of a diseased fowl (A) killed a rabbit in 48 hours with

only slight oedema. From the oedema and blood cultures were obtained. Subcutaneous injections of one loopful of a culture obtained from the blood of this rabbit had no effect on two fowls and a pigeon. (2) A rabbit, which received one loopful of a 48 hours' agar culture derived from rabbit No. 1, died in 48 hours without well-marked oedema. Pure cultures were obtained from the blood. (3) A rabbit inoculated subcutaneously with ·25 c.c. of oedematous fluid from bull No. 2 died in 12 days. The autopsy showed extensive oedema and 200 c.c. of yellow fluid in the peritoneum. Cultures were obtained from the fluid, oedema, and blood. (4) A rabbit which received ·5 c.c. of diphtheria antitoxin, followed next day by a loopful of a serum culture from rabbit No. 1, died in 15 hours, with lesions similar to those found in rabbit No. 1.

IV. *Cats, Dogs, and Rats* proved resistant to subcutaneous inoculation with emulsions of pseudo-membranes and cultures from various sources.

Holmes apparently considers that this is an instance of a true diphtheria outbreak occurring in children, cows, and fowls simultaneously, but the organisms obtained from all sources do not agree with diphtheria bacilli in their pathogenic properties, and they seem to differ to some extent in their morphology and cultural characters. Although a rabbit was protected by antitoxin from the bacilli obtained from one of the children, yet the septicaemic condition produced by the inoculation of minute doses into fowls, rabbits, bulls, cats, dogs, and rats differs entirely from the lesions produced in these animals by the injection of true diphtheria bacilli. Rats indeed are well known to be extraordinarily resistant even to enormous doses of virulent diphtheria bacilli. The bacilli derived from the infected birds, although producing a similar condition in bulls and rabbits, were almost non-virulent for fowls, and totally without effect on pigeons, cats, dogs, and rats. Moreover, a rabbit was not protected in any way by antitoxin.

Summary of Chapter VII.

The subcutaneous inoculation into a guinea-pig of a pure culture of a virulent diphtheria bacillus causes the death of the animal within three or four days with characteristic lesions, namely, a gray necrotic pseudo-membranous focus at the site of inoculation, more or less extensive gelatinous oedema, pleural effusion, and more or less well-marked congestion of the viscera, especially the suprarenal capsules. The bacilli are found in large numbers at the site of inoculation, and can sometimes also be recovered from the blood and organs. Intraperitoneal inoculations also cause the death of the animals. Pseudo-membranes may be produced by inoculation of the mucous surfaces, and paralyses may be caused by the injection of dilute toxins, or living cultures and antitoxin. Rabbits, dogs, and cats are also susceptible. In the latter fatty degeneration of the cortex of the kidney is a marked feature of diphtheritic intoxication (Klein). Local lesions are produced in cows by the subcutaneous injections of cultures, and, according

to Klein, vesicular lesions also occur on the teats. The same observer thinks that the bacilli pass into the milk. These results have not been confirmed by other workers. Rats are very resistant to the diphtheria bacillus and its products. Birds (fowls, pigeons, etc.) can be killed by subcutaneous and intramuscular injections of diphtheria bacilli, and false membranes may occasionally be produced in the trachea by the application of cultures to the abraded surface.

The disease has been proved to occur naturally in the horse, and lesions associated with the presence of virulent diphtheria bacilli have been described in the cow. In each instance human subjects have been infected from these sources. Both cats and fowls have frequently been regarded as carriers of the disease, but the bacteriological evidence in support of these statements is unsatisfactory. Instances of natural infection amongst other animals are unknown.

Bacilli closely resembling diphtheria bacilli in many of their characters have been found in dogs, guinea-pigs, rats, fowls, turkeys and pigeons.

CHAPTER VIII.

THE MODES OF INFECTION IN MAN.

Transmission of the bacilli from patients, directly and through infected articles. Transmission of bacilli by persons suffering from atypical forms of diphtheria, Nasal catarrh, Fibrinous rhinitis, Tonsillitis, Otorrhoea. Transmission of diphtheria by healthy contacts. Mode of transmission from unrecognised cases and healthy contacts. Infection through infected articles, food substances, milk, animals, birds, air, dust, soil, drains and sewer-gas. Laboratory infection. Summary.

For some years before the recognition of the diphtheria bacillus it had been almost universally admitted that one of the most common modes of dissemination of diphtheria was from diseased persons to others. The discovery of the diphtheria bacillus led to a more accurate knowledge of the means by which the disease is spread. Numerous researches have made it certain that a considerable percentage of those who come into contact with the patients acquire the bacilli, but only suffer from the disease in a modified and clinically unrecognisable form, or remain healthy. Many of these persons probably owe their immunity to the presence of antitoxin in their blood (see p. 41). It is these persons who are the chief factors in the spread of the disease, since they are frequently neither isolated nor suspected, and in their turn distribute the infective agents to others. Statistics have already been given (pp. 181—188) showing the percentage of such infected healthy persons found amongst various classes of "contacts," and the methods by which the bacilli pass from one individual to another will only be discussed here.

(1) *The Direct Transmission of Bacilli from Patients to Healthy Individuals.*

Patients suffering from diphtheria frequently cough out with considerable force small pieces of membrane or masses of mucus or saliva containing bacilli, which may be deposited on the mucous surfaces or

skin of those in attendance on them. Numerous instances of the transmission of the disease in this manner are given in the literature, but need not be quoted here. On several occasions also the attempt to clear the passages by applying the lips to a tracheotomy tube and removing the block by suction has resulted in the infection of the operator.

(2) *The Transmission of the Bacilli from Patients by Infected Articles.*

(*a*) It has already been shown that diphtheria bacilli may remain alive for a considerable length of time when enclosed in membranes or masses of expectorated mucus even after drying (p. 171). Park (1892) for example was able to demonstrate the presence of living diphtheria bacilli in "a bit of membrane no larger than a pin's head four months after its removal from the throat." Such fragments of membrane are obviously a source of continued danger. Linen and other articles soiled by the expectoration and discharges of diphtheria patients have also frequently conveyed the disease to other persons (Park and Beebe, 1895, p. 49).

Examples of such instances when occurring within a short time need scarcely be quoted. Although bacteriological evidence is lacking in some of them, several highly interesting cases have been cited by various observers showing the possibility of infection by soiled articles long after the date of their infection.

Carstairs (1901) recounts the case of a father and son who were cornet players being attacked by diphtheria. The instrument was put away. A younger member of the family having found the cornet played it and developed diphtheria in a week. There had been no case in the district for eight weeks previously. Bugbee (1904) states that certain library books, which had been used by a diphtheria patient the day before the appearance of the typical clinical signs of diphtheria (confirmed by cultures), were wrapped in brown paper and returned to the library. These were put in the cellar and fumigated. Eleven months later one of the committee found the parcel and opened it, and seven days later developed diphtheria. Warry (1895) reported an outbreak in which 23 (out of the 26) cases occurred amongst 43 adult females occupying a work room. He thinks that the speaking tubes, which were largely used, were instrumental in conveying the disease.

There is also some experimental evidence to show that diphtheria bacilli may remain alive on naturally soiled linen for a considerable

time and even in dust and on hair, etc., although apparently not protected by dried secretions.

Trevelyan (1900) gives an instance in which diphtheria bacilli were cultivated from a handkerchief 11 weeks after it had been used by a child suffering from diphtheria. Wright and Emerson (1894) once cultivated virulent diphtheria bacilli from a brush used for sweeping a ward, and on three occasions isolated virulent bacilli from the dust clinging to the shoes of nurses. They also found a diphtheria bacillus of low virulence in one out of four cultures made from the hair of nurses, but numerous cultures made from the sweepings of the floor, scouring clothes, skirts of nurses and patients, finger nails, etc., were negative as regards the presence of the diphtheria bacillus.

Weichardt (1900) examined objects surrounding diphtheria patients by means of damp swabs, 50 samples being taken from the sick room and 250 from other parts of the house. Diphtheria bacilli were found three times, all from objects directly soiled by the patient, *i.e.*, a bottle, a neck cloth, and a spot on the carpet within ·5 metre of the patient's mouth. Hill (1902), by means of damp swabs, also found non-virulent diphtheria bacilli on two articles (toy and handkerchief) closely connected with the patients after the latter had been officially released from isolation following upon two consecutive negative bacteriological examinations. Fourteen doubtful, non-pathogenic bacilli were obtained from sheets, pillows, mattresses, bed frame and floor amongst the 331 other swabs examined. Three hospital wards, all occupied at the time of examination, were also investigated, using 197 swabs, with negative results. He mentions that the collector was careful to select exposed surfaces on sheets, pillows, furniture, walls and floor near the patients. At least two swabs, usually more, were rubbed on each area selected.

In order to test the efficiency of swabs for removing *B. diphtheriae* from wooden and cotton surfaces, on which bacilli had been dried, the following experiments were made. The wooden surface of an old varnished box and some new factory cotton were sprayed with the same emulsion of diphtheria bacilli made by suspending a 24 hours agar culture in water, and both were set drying in a protected place in diffuse daylight. The swabs were moistened in sterile water before use. A different area of each surface was tested each different time tests were made, but all swabs used in any one test were taken from the same area.

The order of taking the swabs, rubbed different numbers of times on the same area, is shown on the second and third days

in the following tables, which summarise the results of these experiments:

Results from cotton.

Area tested	Time of drying	No. of swabs	How taken	Result: Positive	Negative	Contaminated
No. 1	3 hours	2	Rubbed repeatedly	0	2	0
,, 2	1 day	2	Each rubbed 8 times	2	0	0
,, 3	2 days	1st set 2	,, ,, 1 ,,	2	0	0
,, 3	2 ,,	2nd ,, 2	,, ,, 2 ,,	1	0	1
,, 3	2 ,,	3rd ,, 1	,, ,, 4 ,,	1	0	0
,, 3	2 ,,	4th ,, 1	,, ,, 6 ,,	1	0	0
,, 4	3 ,,	1st ,, 1	,, ,, 6 ,,	1	0	0
,, 4	3 ,,	2nd ,, 1	,, ,, 4 ,,	1	0	0
,, 4	3 ,,	3rd ,, 2	,, ,, 2 ,,	2	0	0
,, 4	3 ,,	4th ,, 2	,, ,, 1 ,,	2	0	0
,, 5	8 ,,	6	,, ,, 1 ,,	0	4	2
		22		13	6	3

Results from wood.

No. 1	3 hours	2	Rubbed repeatedly	2	0	0
,, 2	1 day	2	Each rubbed 8 times	1	1	0
,, 3	2 days	1st set 2	,, ,, 1 ,,	0	2	0
,, 3	2 ,,	2nd ,, 2	,, ,, 2 ,,	2	0	0
,, 3	2 ,,	3rd ,, 1	,, ,, 4 ,,	1	0	0
,, 3	2 ,,	4th ,, 1	,, ,, 6 ,,	1	0	0
,, 4	3 ,,	1st ,, 1	,, ,, 6 ,,	0	1	0
,, 4	3 ,,	2nd ,, 1	,, ,, 4 ,,	0	1	0
,, 4	3 ,,	3rd ,, 2	,, ,, 2 ,,	2	0	0
,, 4	3 ,,	4th ,, 2	,, ,, 1 ,,	2	0	0
,, 5	8 ,,	6	,, ,, 1 ,,	0	5	1
		22		11	10	1

From this table it is seen that, omitting the eighth day results, attempts to obtain positive results from the cotton were successful in three out of the first four tests, while some swabs were positive in each of the first four tests made from the wood.

(3) *The Transmission of the Bacilli by Persons suffering from Atypical Forms of Diphtheria.*

That diphtheritic infection may pass from patients to healthy individuals by the means which have been mentioned has long been recognised, and therefore scarcely needs illustration. The more interesting question, however, of the danger to the community arising

from the presence amongst the normal population of more or less healthy infected individuals must be discussed at some length. Many of these persons have probably been infected from patients, before the development of clinical signs, by the means by which they themselves spread the infection. These more or less healthy individuals, who have become infected with diphtheria bacilli and are therefore possible factors in spreading the disease, may be conveniently divided into three groups according to the classification of Watson Williams (1905).

Group 1. "Patients who afford none of the usual clinical indications of diphtheria, are not definitely ill, and yet are found to be anaemic, have increased pulse frequency, are poorly, in association with nasal catarrh, membranous rhinitis, faucial redness, and slight subacute tonsillitis, otorrhoea, sores, etc., which on bacteriological examination prove to be diphtheritic."

Group 2. "Cases with any of these diphtheritic lesions, but with no general symptoms of ill health." (Group 3, see p. 314.)

That persons belonging to group 1 are capable of spreading the disease, and even of causing considerable outbreaks, has been well recognised.

Owing to the fact that many of the accounts do not differentiate between these groups it is at present difficult to determine, whether the members of group 2 are less dangerous than those of group 1, or not. Williams considers that they have less frequently given rise to epidemics.

Some noteworthy examples are, however, quoted in the following pages, which either gave rise to a considerable number of cases, or caused infection after long periods of time.

(*a*) *Nasal catarrh.*

Cobbett (1901, p. 231) records as follows a remarkable instance of an outbreak traced to a child at first apparently suffering from nasal catarrh. "While the actual origin of the outbreak has not been cleared up, there is no doubt about the way in which the infection became distributed: on October 23rd, G. N. was visited among other infants attending this school. He was found to be having tea with his brother and sister, and was in very good spirits, though he looked rather pale, and appeared to be suffering from nasal catarrh. His mother said he had had a 'stuffy cold in his head' for about three weeks. During this time he had been regularly attending the school. Bacteriological

examination revealed diphtheria bacilli in great numbers in the nasal discharge, and after his removal to the hospital membrane was seen in his nose. His father, mother, sister and brother, all the members of this family, were found to have diphtheria bacilli in their throats. From three of these (including G. N.) cultures were isolated and proved to be virulent. No less than seven of the nine male members of his school class suffered from diphtheria before October 23rd."

Burnett (1900) also gives an instance of the discovery in the nasal discharge of a boy, supposed to be suffering from a severe cold, of diphtheria bacilli, which lingered for three months. After two negative bacteriological examinations he was allowed to return to school. Shortly afterwards a fatal case of diphtheria occurred, and on examination diphtheria bacilli were again found in the boy's nose.

Park and Beebe (1895, p. 41) mention an interesting case. "A child was admitted into a hospital ward in an anaemic condition and with a chronic coryza. Five days later four children in his neighbourhood developed diphtheria. Two of these died. In seeking the cause of the diphtheria, suspicion was directed to the child by a slight nasal discharge. Bacteriological examination showed this secretion contained many diphtheria bacilli. On further examination it was found that the child came from a family in which three weeks before there had been a case of diphtheria."

Newsholme (1904) quotes the following example. F. T., aged four, developed diphtheria on November 16th, and it was found that on November 7th, his brother I. T. had suffered from headache followed by nasal discharge. He had continued to attend school until the 18th, and infected three other children in the same class, who failed with the disease, two on November 14th, and one on the 19th. Diphtheria bacilli was found in I. T.'s nose.

(*b*) *Fibrinous, or membranous rhinitis*[1].

Cobbett (1901) observes that "virulent diphtheria has been but seldom observed to be contracted from contact with a case of membranous rhinitis," and some observers have considered that the virulence of the bacilli isolated from such cases is low, and that cases of this disease are apt to give rise to others of the same kind. Some statistics on this latter point are given elsewhere (Chapter X)

[1] Cobbett's case quoted under the heading of Nasal Catarrh, although no membrane was at first noticed, perhaps ought to be more properly included amongst this series.

which show that a very large proportion of the bacilli isolated from these cases are fully virulent, and several cases of clinical diphtheria occurring through contact with persons suffering from membranous rhinitis have been recorded.

Dowson (1895) gives a very interesting example with the following history. "L. H. and S. H. went to a children's party on December 17th, 1894, at the house of a lady who had a tendency to suffer from sore throat, and who about three weeks before the party was under the care of a medical man, the father of L. H. and S. H., for a somewhat indefinite ailment of the throat, attended with slight difficulty in swallowing. Both children were kissed by this lady, and on December the 19th, L. H., a healthy boy of 4½ years, had a slight nasal catarrh, but was apparently quite well otherwise. On December 28th, his right nostril was noticed to be blocked by a tough white membrane which, when detached, did not cause bleeding, although an abraded vascular surface was exposed. On January 2nd, S. H., the sister, aged six years, was noticed to be affected in a similar manner in the left nostril." Virulent diphtheria bacilli were isolated from these membranes. "At the end of two months, so very few bacilli, and these chiefly involution forms, could be recovered from the children's noses by culture methods, that they were considered harmless to others, and the fairly strict quarantine, which had been observed, was removed. Active local antiseptic treatment had been employed during the whole time. At the end of May, the mother of the children, after playing with and kissing the boy to a quite unusual extent, complained of sore throat, which on inspection proved to be a membranous pharyngitis presenting all the appearances of true diphtheria. This was confirmed by inoculating a culture tube from the membrane, the resulting growth being almost a pure culture of the diphtheria bacillus. It was at first sight thought that the mother's attack was due to some oversight in disinfection, but an examination of the children's noses showed that membrane resembling that first observed, was again present." Diphtheria bacilli were again found.

Ravenel (1895) gives an account of several children, who became infected from a child suffering from membranous rhinitis, produced by virulent diphtheria bacilli. The first child, who remained quite well except for a sensation of discomfort in the nose, had himself been infected by a child who only complained of a sore throat. The former gave rise to membranous rhinitis in three children, one of whom later developed pharyngeal diphtheria, and to two cases of the latter disease.

Cultures made from all these cases showed diphtheria bacilli. Wolff (1905) also quotes a case of true diphtheria apparently infected from a case of membranous rhinitis.

On the other hand, a case of membranous rhinitis has not infrequently been observed to give rise to another of the same kind. The cases just quoted from Ravenel are examples, and the same observer also gives another instance in which a mother and three children all suffered from this complaint. Abbott (1893) found two children affected in the same family, and Concetti (1892) obtained in two cases a history of direct infection from one to the other. Since that time several other instances of two or more members of the same family suffering from membranous rhinitis have been described. Lieven (1891) reported a case of membranous rhinitis from which he obtained an organism that when introduced into the noses of other children by means of tampons caused a similar condition in them.

(*c*) *Tonsillitis.*

Bissel (1902) relates a case in which a child, who had had a mild sore throat two months previously, went to pay a visit. Diphtheria occurred in the household visited, and was transmitted by this child as far as could be ascertained. He adds that many such fully authenticated cases have occurred in the city of Buffalo. Berry (1900) gives an account of a prolonged outbreak apparently transmitted through children suffering from sore throats. In the London Orphan Asylum a case of diphtheria was introduced amongst the girls (200) on February 25th. A series of mild sore throats followed till March 27th, when another case of diphtheria occurred and three children with sore throats were found to be harbouring diphtheria bacilli. These were isolated. Further cases of sore throat followed till April 30th, when five cases of diphtheria occurred. At this time there were 76 cases of sore throat. Dr Washbourn examined all the children with abnormal conditions of the throat and found diphtheria bacilli in 17. These were isolated and not allowed to return to school until free from the bacilli. From this time no further cases of sore throat or diphtheria occurred. Newsholme (1904) gives an instance of a child who was ill for one day with sore throat and headache. Five days later his sister developed diphtheria. This led to a swab being taken from his throat, from which diphtheria bacilli were cultivated.

Goodall (1896) quotes a case in which diphtheria was apparently

transmitted by a patient several months after recovery from a mild attack. A nurse had a very mild attack in January and was isolated for several weeks because the bacilli persisted. When free she was put in charge of a ward of mixed diphtheria and scarlet fever. In the autumn she went home to a village in Oxfordshire, where there had been no diphtheria for a long period, and almost immediately her sister and niece developed diphtheria.

(*d*) *Otorrhoea.*

Newsholme (1904) gives an interesting example of infection through a case of otorrhoea. A baby, nine months old, was apparently infected from its sister who developed typical diphtheria. "Except for the ear trouble the baby remained perfectly well, but infected another child, who suffered from pharyngeal diphtheria. At the time the other child became ill a pure culture of diphtheria bacilli was obtained from the ear discharge about a month after its commencement.

The writer (Graham-Smith, 1904) has also observed a small outbreak in which two persons were attacked with clinical diphtheria, and five became infected with virulent diphtheria bacilli, apparently by contact with a patient suffering from otorrhoea in whose discharge virulent diphtheria bacilli were found.

(*e*) *Other lesions.*

Newsholme (1904) quotes a case in which enlarged cervical glands were the only clinical evidence of a pathological condition of the throat. N. W., aged three, and his brother S. W., aged two, developed diphtheria on October 20th and 25th, respectively. Inquiry into these cases led to the discovery that the brother G. W., aged five, had come home from school with enlarged glands on October 7th. Diphtheria bacilli were found in his throat on October 25th.

(4) *The Transmission of Diphtheria by Healthy Contacts.*

Williams' third group consists of healthy infected contacts or "persons who present no local lesions and no departure from normal health, but in whom diphtheria bacilli have been found by culture tests." Williams says of this group that "it has yet to be shown that such infected contacts are liable to spread diphtheria or are actively infectious till they develop local symptoms." In support of this view

he quotes instances in which healthy infected contacts were allowed to mix with the non-infected persons in institutions without ill effects. Diphtheria, however, is usually most prevalent at seasons of the year when "colds" are common, consequently a member of this group may at any time by developing a "cold" be converted into a member of group 2. Even if Williams' opinion is correct, it is therefore almost as necessary to guard against the possibility of the spread of the disease by members of group 3 as of group 2. There are, however, several examples to be found in the writings on this subject, which indicate that perfectly healthy infected contacts are capable of spreading the disease, and careful observation could probably multiply them. The writer has come across seven instances in which there could be but little doubt that the disease had been conveyed by such persons, and some in which the proofs were convincing. In one instance a girl aged 13 was admitted into a general hospital for an operation on the knee. Shortly after her admission a small outbreak occurred in the ward owing to the introduction of an unsuspected case. On the day before her discharge cultures were obtained from this girl, which showed the presence of a few colonies of diphtheria bacilli, which were later proved to be virulent. On the urgent request of her parents, however, she went home to a small village in which no cases of diphtheria had occurred. About a fortnight after her return, diphtheria broke out amongst the members of her family, of whom three suffered from the disease. Although the girl remained in perfect health subsequent cultures showed the presence of numerous colonies of diphtheria bacilli.

Bugbee (1904) gives the following example: A young woman, six weeks after an attack of diphtheria, came to reside at Brooklyn with a family consisting of two adults and two children. Six days later one adult and one child developed diphtheria. The grandmother of the healthy child came to attend to it and was in the house for four weeks, but did not see the diphtheria patients. When the grandmother returned home to Vermont two members of her family developed diphtheria within a week. From her throat a pure culture of diphtheria bacilli was obtained.

Park and Beebe (1895) quote an instructive case. "In a family of eight children one sickened of diphtheria and a second child, a baby, was sent to a neighbour. The next day cultures showed that this baby, as well as two of the other children, all of whom were apparently healthy, were infected with diphtheria bacilli. The three apparently healthy but infected children, as well as the sick one, were at once

quarantined, but already one of the family, to which the baby had been sent, had contracted diphtheria from it."

Hutchens (1906) records a noteworthy example of the conveyance of diphtheria by a perfectly healthy contact. "One of the pupils at a private boarding school contracted diphtheria. As soon as the true nature of the disease was recognised, the throats of all the inmates of the establishment, some thirty in all, were examined. Diphtheria bacilli were found in the cook's throat and in no one else's. On being questioned she admitted having visited a neighbouring town some three or four days previously, and, while there, had been in contact with a case of diphtheria. These statements were investigated and found to be correct. There was no diphtheria in the town in which the school was situated, and the cook herself was in perfect health."

White (1901) also recounts an instance of the spread of diphtheria by perfectly healthy contacts. Four persons, continually exposed to a child which harboured diphtheria bacilli, were examined three months after the latter had recovered, and diphtheria bacilli were found in the throats of two of them. About this time two children were exposed to these persons for a day or two, and one of them on going home immediately developed diphtheria. No other source of infection could be traced.

Cuno (1902) investigated an outbreak which occurred first in one and then in another ward of a hospital. On careful examination it was discovered that one nurse had virulent diphtheria bacilli in her throat, and the outbreak was found to have followed her removal from the one ward to the other.

Newsholme (1904) also gives an instance of the spread of the disease by a healthy infected contact. F. W., aged six, attended until March 13th a school in which cases of diphtheria were occurring at the time. She was kept at home because she had ringworm, but is stated to have had no cold or sore throat. On March 19th, her sister E. W., aged $4\frac{1}{2}$, failed with diphtheria. E. W. had not been attending school, and had been kept strictly indoors owing to a recent attack of chicken-pox.

Although no bacteriological evidence is mentioned, the following case related by Newsholme (1904) is of interest. During an outbreak of diphtheria a girl, although she sat next to another who attended school for several days while suffering from an attack of unrecognised diphtheria, remained perfectly well. Within a few days a young man who lodged at the former's house developed diphtheria. He had only

one meal in the house which was brought up to him by this child or her sister.

One of the most interesting cases of the transmission of diphtheria by non-sufferers is that recorded by Peck (1895). The first case of the series was that of R. H., aged 45, who lived in house A, and began to be ill on October 12th, 1894. "The case was typical and was followed by double drop-wrist. No other persons in this house suffered from sore throat." The next case occurred in house B, which was half a mile distant from A. "There was no communication between the houses till December 26th, when the occupier began to work for R. H. Case 2, E. J., aged $7\frac{1}{2}$ years, had a sore throat on or about January 2nd, 1895. The case was typical and followed by paralysis of both legs. The patient died of cardiac syncope. Case 3, E. J., aged four years, began to be ill on February 6th. This was a typical case and resulted in death from asphyxia. Case 4, J. J., aged 12, had a sore throat on February 16th. The case was typical and followed by dimness of sight and strabismus. Bacteriological examination of cultures showed the presence of large numbers of diphtheria bacilli." All these persons were members of one family. The next case occurred at house C., which was $1\frac{1}{4}$ miles distant from A and B. "R. J., aged 18, brother of the last three patients, worked here from January 15th. His mother stated that he was at home for a few minutes on the days his sisters were buried, but at no other time. He himself admitted having been at home on each Sunday to change his shirt, which he did in the room occupied by the patients. He stated that he did not suffer from sore throat at any time, but bacteriological examination showed typical diphtheria bacilli in large numbers to be present in his throat. There is no history of his ever having had diphtheria. Case 5, J. C., aged 18, began to be ill on February 26th. He worked on the same farm as the last youth, and slept with him. He went home to house D on February 27th, being then 'full of cooth,' and suffering from sore throat and unable to work. Typical diphtheria bacilli were present in large numbers on March 16th." House D was nearly two miles distant from all the other houses. "Case 6, J. C., aged 13, slept with the last patient, his brother. On March 8th he was seen for the first time by his medical attendant and was then dying. No bacteriological examination was made. Case 7, E. C., aged three, sister of the last two patients, began to be ill on March 12th. The case was a typical one, and an abundance of typical diphtheria bacilli was found on bacteriological examination."

"House B was probably infected by the father of the family, who himself did not suffer from the disease. His son R. J. did not suffer from the disease, though he was proved to have its germs in his throat, but he was the means by which it was carried to house C, whence it was taken to house D."

Cases of infection carried by patients long after they have recovered from the disease, and appear to be in perfect health, have frequently been recorded. Such persons are in the same condition as healthy individuals carrying virulent diphtheria bacilli and might reasonably be included in this group, though in most accounts they are separately considered.

It is only those persons who are infected with virulent diphtheria bacilli, who are apparently capable of transmitting the disease, since there is little or no evidence to show that persons harbouring non-virulent diphtheria bacilli can infect others with virulent bacilli.

The Mode of Transmission of Bacilli from unrecognised Cases and healthy infected Contacts to other Persons.

Instances occur, probably not infrequently, in connection with unrecognised cases, and healthy infected contacts, in which the bacilli are conveyed to others in saliva through coughing or sneezing. The kissing of babies and children as a means of spreading the disease has been particularly insisted on by Hewlett and Murray. These methods of infection can only affect a small number of very close contacts, mostly limited to the families or intimate friends of the infected children.

In schools, however, the passage of sweets, pencils, pens, slates, etc., from one child to another, and especially the habit children have of placing their fingers, and such articles as pencils in their mouths, explain the rapid spread of infection in such institutions; and the absence of these habits in adults may to some extent account for their relative immunity from the disease.

Some remarkable examples of transferences by these means are given out of the large numbers which might be quoted:

Cobbett (1901, p. 247) gives two examples. "The day before B. C. was taken ill he had spent the evening with some neighbours. Four of these were examined for diphtheria bacilli with the result that they were found in two boys of 16 and 20, but not in a baby, nor yet in a sister of 14. On further inquiry it was discovered that B. C. had played with one of these boys at parlour cricket and each had taken

it in turn to score with the same pencil, which doubtless often found its way into their mouths. It is quite clear then how the bacilli found their way from B. C. to this boy. From him they easily found their way to his brother, for the two boys slept in one bed." Cobbett (1901, p. 232) also traced the spread of the disease amongst certain school children in a class to the hours during which slates were used. This class consisted of nine boys and seven girls. It was notable that while seven of the nine boys of this class suffered from diphtheria, only one of the seven girls in it was affected, and one other was discovered to be harbouring a virulent diphtheria bacillus. For almost all the lessons the boys and girls of this class were mixed indiscriminately, and according to their school mistress they played together out of school hours. The only explanation of the unequal incidence of the disease upon the boys and girls was that twice a week the girls were separated from the boys to do needlework, whilst the latter had a drawing lesson. It can scarcely be doubted that it was during this drawing lesson, when slates were in use, that infection was distributed. One of the boys attended the class for three weeks while suffering from unrecognised nasal diphtheria.

In this connection Bond (1898) remarks that he has ascertained that in certain schools each child does not have its own slate, and that as a means of cleaning their slates licking is common.

Wesbrook (1900) remarks that "in watching children at play it does not take long to be convinced of the almost innumerable opportunities afforded for the exchange of the bacterial flora of mouths and noses," and later states (1905) that "the experience of Minnesota would seem to point decidedly to the conclusion that diphtheria infection is transmitted usually by almost direct exchange of the flora of the nose and mouth."

The Conveyance of Infection by other means than Personal Contact.

As compared with personal contact the other means by which diphtheria may be spread are, with the exception of infection through milk, of little practical importance. Infection is recorded to have been conveyed by infected articles, milk, food substances, animals, and birds, and by the agency of air currents, drains, and soil.

(1) *Infected articles.* Instances of infection by means of infected articles which may pass from mouth to mouth, practically amounting

to direct transmission, as well as examples of the conveyance of diphtheria by musical instruments, etc., long after their infection, have already been quoted. Experimental evidence of the cultivation of diphtheria bacilli from articles closely connected with patients, as well as evidence showing that diphtheria bacilli may remain alive for long periods in a dried condition, have also been given.

Many other articles may become soiled by diphtheritic excretions or saliva containing virulent bacilli and convey the disease, but attention ought to be especially directed to speaking tubes and to cups or glasses from which several individuals drink in succession, more particular those attached to public fountains.

(2) *Food substances.* Experimental evidence of the time diphtheria bacilli may remain alive and retain their virulence in some food substances, including water, has already been given (p. 172). Certain of these substances, such as sweets and fruit, which often pass from one child to another, frequently convey infection in the same manner as pencils, etc. Park and Beebe (1895, p. 51) traced a group of cases to a sweet shop. "The child of a man who kept a candy-store developed diphtheria; there were four other children in the family, and these were in no way isolated from the sick, yet none of them developed diphtheria; but the children who bought candy at the store, and other children coming in contact with them at school, developed diphtheria. The secondary cases ceased to develop as soon as the candy-store was closed."

No widespread epidemic seems to have been reported which was spread by the agency of any infected food substance except milk. Vincenzi (1898), however, on examining the holy water in churches during an epidemic found amongst other bacteria, diphtheria bacilli. He proved their identity by culture and virulence tests.

(3) The evidence relating to the dissemination of the disease by *animals* and *birds*, is fully given elsewhere (Chapter VII).

(4) *Air-borne infection.* Welch (1891), writing 15 years ago, said that "diphtheria is one of the infectious diseases the germs of which may be taken into the body by the inspired air," and up to the present time examples are occasionally quoted of apparent air-borne infection.

It must, however, be pointed out that diphtheria bacilli have seldom, if ever, been found in the air of wards or rooms occupied by diphtheria patients, in the few attempts which have been made to investigate this problem.

Wright and Emerson (1894), although they found diphtheria bacilli on shoes, brushes, etc., twice examined the air of the ward with negative results. Sudeck (1898) exposed plates in rooms, some occupied by healthy persons and others by diphtheria patients, and obtained some colonies of diphtheria-like bacilli, but never found true diphtheria bacilli.

Cobbett (1904) says he has been unable to find diphtheria bacilli on plates exposed in diphtheria wards except in situations in which they could easily be contaminated by the patients, and Hill (1902) also in a large number of experiments only appears to have found bacilli in similar situations (see p. 308).

Though it cannot be denied that air-borne infection may occur, both the experimental and the more recent clinical evidence point to its being a very rare mode of dissemination.

(5) *Dust.* That the dust found on the floors of hospital-wards may contain virulent diphtheria bacilli has been proved by the researches of Wright and Emerson (p. 308), and the power of the diphtheria bacillus to survive for long periods in dust on dry surfaces has been experimentally demonstrated by Germano (1897), Hill (1902) and others.

The possibility therefore of infection by means of floating particles of dust stirred up by sweeping, or otherwise, cannot be denied. Conclusive evidence of infection by this means has not been obtained, though some instances are given in the literature, which might be attributed to this cause, as, for example, of persons developing the disease after moving into houses lately occupied by patients. Some of the cases attributed to aerial infection may also have been due to dust.

(6) *Soil*[1]. References are occasionally to be found relating to the origin of diphtheria from polluted soil, but very little evidence is to be found in support of this view, although Leighton (1901) has shown experimentally that diphtheria bacilli can remain alive in moist warm clay for 18 days. Sharp (1896) stated that he found organisms morphologically like diphtheria bacilli and having similar colonies in two soils obtained from a locality in which diphtheria existed. No further tests to prove their identity appear to have been made.

(7) *Drains and sewer gas.* The belief is extremely widely held, particularly in Great Britain, that sewer gas is the predisposing cause of diphtheria, and scarcely a case occurs but it is attributed to "drains"

[1] The condition of the soil in regard to dampness and the exhalation of gases may affect the incidence of diphtheria by lowering the general health (see p. 69).

by the friends, and frequently by the medical men. Individual cases and even outbreaks have been frequently attributed to decaying manure and refuse heaps.

For example, Sutton (1894) states that in the parish of Upchurch large quantities of refuse from London dust-bins are brought by barges, and deposited on the quay. "These masses lie mouldering for weeks and the noxious fumes are carried in the direction favoured by the wind." At one time five cases of diphtheria occurred, and Sutton considered that these were infected by the foul gases. He concludes with the following observations:

"After due inquiry as to the origin of the first and subsequent cases, I came to the conclusion that these cases of diphtheria originated in the deleterious emanations generated in such immense volumes and were air-borne, especially as there was an utter absence of proof, or even suspicion, of direct personal contagion and no evidence pointing to schools as the cause. I am fully of the opinion that this disease originates with insanitary conditions, especially foul nuisances."

On the other hand Delépine (1904) recently investigated an outbreak attributed to an effluvium nuisance from a tannery, but found on careful inquiry and examination that the infection had been brought into the locality by a diseased person.

Many of these illnesses, which are mistaken for diphtheria, and which apparently result from the inhalation of foul gases, are, however, not associated with diphtheria bacilli. Diphtheria bacilli have never been found in drains or sewer gas, or in refuse heaps, and there is no bacteriological evidence to show that the emanations from the latter can originate true diphtheria; nor is there evidence that bad drains and insanitary environment can ever convert non-virulent into virulent bacilli. Shattock (1898) experimented on this question and found that it was impossible to raise the virulence of lowly virulent bacilli by cultivating them in sewer air, even after two months.

Hutchens (1906) gives an interesting example of apparent infection due to a bad system of drainage, which was subsequently proved to be caused by infected milk (see p. 325).

(8) *Laboratory infection.* Accidental infections in laboratories have from time to time been recorded, but are very rare.

Milk Epidemics.

In 1878 Power[1] brought forward evidence that diphtheria might be spread by means of milk. Between 1878 and 1882 several epidemics

[1] *Local Government Board Report for* 1878.

occurred in which the milk appeared to be the principal source of infection. The first milk epidemic following the discovery of the diphtheria bacillus occurred in York Town and Camberley in 1886 and was investigated by Power[1], and Klein's experiments, already quoted (p. 282), were undertaken in connection with this outbreak. Several outbreaks have since been recorded in which the milk supply has been the only source of infection in common amongst the patients, but in the great majority bacteriological evidence of the presence of the diphtheria bacillus in the milk, or in the throats of the milkers or their families, or in any lesions on the cows' udders is wanting. References[2] only to the most striking of these outbreaks are given, although the reports of some of them are extremely convincing and show that the outbreaks came to an end when the infected milk supply was stopped.

Some typical examples of milk epidemics are here summarised in which some bacteriological evidence of diphtheritic infection amongst the milkers was obtained, as well as all those outbreaks in which diphtheria bacilli were actually isolated from the milk.

Appleget (1893) describes a severe epidemic apparently due to infected milk at Hightown, a place of 2,000 inhabitants. Although no bacteriological proof of the contamination of the milk is produced the outbreak has several features of interest. In the first week of the outbreak 28 persons were attacked, and of these 11 died. Milk was supplied to the town by six dairymen but the disease was confined to the customers of one of them. Investigation on the fifth day of the outbreak showed that the boy who washed the cans at this dairy

[1] *Local Government Board Report for* 1886 (pp. 311–326).

[2] The following outbreaks are good examples of diphtheria apparently spread by milk.

Little Horton, Bradford, 1879 (H. Butterfield, *Brit. Med. Journ.*, 19. vi. 1880, vol. i. p. 953).

Kilburn and St John's Wood, 1879 (W. H. Power, *Local Government Board Report*, 1879).

Rugby, 1881 (G. Wilson, *Brit. Med. Journ.*, 3. ix. 1881, vol. ii. p. 415).

Hendon, 1882 (W. H. Power, *Local Government Board Report*, 1884, p. 42).

Devonport, 1882 (H. F. Parsons, *Brit. Med. Journ.*, 5. v. 1883, vol. i. p. 876).

Canterbury, 1886 (Wacher, *Brit. Med. Journ.*, 21. viii. 1886, vol. ii. p. 397).

Melrose and Malden, 1887 (J. S. Clark, *Brit. Med. and Surg. Journ.* vol. ii. p. 100).

Enfield, 1887 (R. B. Low, *Local Government Board Report*, 1888, p. 123).

Croydon, 1890 (Philpot, *Brit. Med. Journ.*, 1891, vol. i. p. 470).

Worcester, 1891 (Thussfield, *Public Health*, 1891–2, p. 130).

Surbiton, 1891 (Coleman, *Public Health*, 1891–2, p. 158).

Glasgow, 1892 (Russell, *Brit. Med. Journ.*, 1892, vol. ii. p. 432).

Limekilns, 1892 (Nasmyth, *Annual Report*, 1892).

Short accounts of most of these outbreaks are given by Swithenbank, H., and Neumann, G. (*Bacteriology of Milk*, 1903, pp. 346–351).

had a sore throat with patches of membrane on the tonsils, and had been ill for several days previously. In one instance the adults in one house obtained their milk from this source, while the children obtained it from another. Two of the adults contracted the disease, but the children escaped. Only two cases occurred outside the village, one the daughter of the dairyman, and the other his niece.

Howard (1897) investigated a more extensive outbreak of diphtheria at Ashtabula in which 100 cases were notified, of which 64 occurred within eight days. These 64 occurred in 49 widely separated houses, and their true nature was proved by the isolation of diphtheria bacilli from many of them. These cases were entirely confined to houses receiving their milk from one source. On inquiry at the dairy it was ascertained that one of the workers had recently suffered from a bad sore throat, and that this patient, while ill, had assisted at the dairy work. At the time of investigation no diphtheria bacilli were found either in the milk or the throat of this person.

Lee (1898) records an outbreak in which, owing to its sudden origin and the impossibility of the patients having come into contact with one another, the milk supply was immediately suspected. On investigating the farm from which the milk was obtained it was found to be in a filthy condition, and streptococci and bacilli morphologically resembling diphtheria bacilli were cultivated from the milk. The latter, however, could not be isolated. Further investigation revealed the fact that the nephew of the dairyman had diphtheria bacilli in his throat, and that a case of severe diphtheria had occurred there three weeks previously.

Biggs (1900) reports a somewhat similar outbreak at Ithica. The milk supply of all the families affected came from one dealer. On inspection all his premises and sanitary arrangements were found to be in good order. This dealer obtained some of his milk from a neighbouring farmer, and in consequence the farm was also visited. Several of the inmates were found to be suffering from sore throats, and cultures taken from their throats showed the presence of diphtheria bacilli. The milk supply was stopped from this farm, and the epidemic came to an end.

The bacteriological evidence in the case quoted by Chase (1900) is even stronger. Two children in a milkman's family developed diphtheria. All the members of the household were examined by cultures, but no diphtheria bacilli were found. Three weeks later diphtheria began to appear amongst the customers. On re-examination of the inmates of the milkman's house three men were discovered to be

harbouring virulent diphtheria bacilli in large numbers. Up to this time two of these men had been employed in milking the cows.

Littlejohn (1900) reports a milk outbreak in Edinburgh and Liberton. At the dairy supplying some of the milk shops in these places a young man was found assisting at the business suffering from unrecognised diphtheria. As soon as the suspected milk was stopped the outbreak came to an end.

Although the bacteriological evidence is not conclusive, an outbreak of diphtheria at Leith recorded by Robertson (1905), apparently spread through the agency of milk, is of some interest. The outbreak coincided with the appearance of ulcers on the teats of the dairy cows, of which 19 out of 45 were affected. When all the diseased cows were isolated the outbreak ceased. Although diphtheria bacilli were found in the throats of most of the patients, swabs taken from the ulcers of the cows only showed a few doubtful organisms whose identity was never proved.

Hutchens (1906) cites an interesting example of the conveyance of diphtheria by infected milk. "A village school was re-opened after the vacation on a certain Tuesday; on the following Thursday evening the schoolmaster's child, aged three years, had a sore throat, which was proved by bacteriological examination to be diphtheria. The following morning three or four children in the village who had attended school on the three previous days also developed the disease. The schoolmaster's child had not himself been to school, neither had he been in contact with the other children. This child only arrived in the village on the Monday night, and there was no diphtheria in the place whence he had come. The school itself had long been notorious for its lack of drainage, and during the vacation a system of drainage had been introduced, but was not completed before the school was re-opened. The surroundings both of the school and of the schoolmaster's house adjoining were most insanitary and offensive. This to the casual observer, was an undoubted instance of diphtheria due to 'insanitary surroundings,' and the outbreak was in fact locally attributed to this cause. Inquiries were now directed to ascertaining whether there were any cases of diphtheria in the neighbouring village. Some four or five were discovered. These could not be explained on the theory of direct contact, as they dated their illness from the same period as the school children. The question of milk supply was considered. It was then found that all the houses in which cases of diphtheria had arisen were supplied with milk from the same farm. A visit was paid to the dairy

farm some miles from the village; it was there found that one of the milkers was complaining of a 'sore throat.' Swabs were taken from his throat and from the throats of all the other people living at the farmhouse. I found virulent diphtheria bacilli in three of these throats. Precautions were taken to prevent those harbouring the bacilli from having anything further to do with the milk, and no further cases occurred in the village. The milk supply from the farm to the village was not interrupted for a single day."

In the following instances virulent diphtheria bacilli were actually isolated from the suspected milk.

Bowhill (1899) isolated virulent diphtheria bacilli from suspected milk during an outbreak at Senghenydd.

Eyre (1899) isolated diphtheria bacilli from the milk supplied to a large school in which diphtheria was prevalent, and proved them to be fully virulent. He obtained them in all the 15 serum tubes sown from the cream obtained after centrifugalisation, but in only five out of 15 sown from the sediment. The examination of this milk was undertaken in connection with an extensive outbreak of diphtheria investigated by Goadby (1900) (see p. 186).

The small outbreak investigated by Dean and Todd (1902) has already been described. The bacilli were isolated from the milk and lesions on the cows' udders, and also from some of the patients, and proved to be virulent (see p. 286).

More recently Ashby (1906) investigated a milk epidemic, and found virulent diphtheria bacilli in the lesions of the cows' teats, but not in the milk (see p. 287).

Klein (1901) obtained a virulent diphtheria bacillus from one out of 100 samples of milk in London. No information could be obtained as to the source of infection.

Summary of Milk Epidemics.

Klein (1890) concluded from his experiments that under certain conditions diphtheria bacilli might be found in the milk as drawn from the udder, and pointed out that in certain outbreaks in which the milk was suspected the cows were suffering from lesions similar to those he obtained experimentally. Other observers have, however, failed to corroborate Klein's results, and the general opinion appears to be that in these cases the diphtheria bacilli are not derived from the cow. Virulent diphtheria bacilli have on four occasions been found in

milk and twice, by Dean and Todd (1902) and Ashby (1906), in the naturally acquired lesions of cows' teats.

The experiments, however, of the former workers showed that the lesions were not due to the diphtheria bacilli.

It has been shown that unsterilised milk at ordinary temperatures is a suitable nidus for the multiplication of diphtheria bacilli (p. 172), and there can be no doubt that milk has been the vehicle for the contagion in certain outbreaks. In most of these it seems probable that it was infected at the dairy or at some other place before reaching the consumers. Dean and Todd point out that a ready means of infection of milk by persons harbouring diphtheria bacilli is the filthy habit of some milkers of spitting on their hands.

Diphtheria-like organisms in Milk.

The occurrence of diphtheroid organisms in milk, recorded by many observers, emphasises the necessity for very careful investigations, including animal inoculations, before a diagnosis of the presence of diphtheria bacilli in milk can be made.

Bergey (1904) isolated from milk, drawn under his observation directly from the udders of the cows, five strains of diphtheria-like bacilli, which in morphology belong to A_1, C_1, and D_1 of Wesbrook's types. They form small smooth gray or yellowish colonies on *serum* like those of the diphtheria bacillus, and on agar the growth is also diphtheria-like. Acid is produced in *glucose broth* and in *milk*. No visible growth occurs on *potato*, and no *indol* is produced. These organisms are non-pathogenic. He also found other organisms which resembled diphtheria bacilli less closely in morphology, produced pigmented growths and also differed in other cultural characters.

Klein (1901, p. 87) isolated from milk a diphtheroid organism, which he named *Bacillus diphtheroides*. This organism resembles the diphtheria bacillus in size, shape and clubbing. It does not stain very readily with the ordinary stains, but shows polar granules and retains Gram's stain. On *serum* it forms small, round granular colonies which at first slightly liquefy the medium and finally make it quite liquid. Growth on *agar* is slow. After three days the colonies are still very small, but after a week they have a dark centre and uneven edges. Very little growth occurs in *broth*. There is no growth on *gelatin*. *Milk* becomes acid and is coagulated.

Intraperitoneal or subcutaneous *inoculations* into guinea-pigs give rise to subacute abscesses containing thick yellow pus from which the organisms can be recovered. After subcutaneous injections the inguinal glands are especially affected, and after intraperitoneal the omentum, pancreas, and perinephric tissue.

McClure (1898) separated from milk a curved, banded bacillus which stained intensely at the poles, but did not retain Gram's stain. Under the microscope

the bacilli had the characteristic arrangement of diphtheria bacilli. On *agar* it produced a considerable white, partly confluent, growth which later became yellowish. It grew rapidly on *gelatin* and produced a grayish white growth on *potato*. *Broth* was made cloudy and a white flocculent deposit was produced. *Milk* was turned acid in 48 hours and coagulated. A *mouse* injected subcutaneously died in 14 hours.

Eyre (1900) isolated from milk five organisms resembling the diphtheria bacillus "The interest of these observations lies in the fact that it would be quite easy to be deceived by the microscopical appearances of any one of these organisms." The organisms could on their cultural peculiarities be divided into three groups which he calls "Diphtheroid 1, 2 and 3."

Diphtheroid 1. *Morphology.* Pleomorphic bacillus of about the size of *B. diphtheriae*, showing segmentation of its protoplasm, metachromatic granules, and early involution forms, characterised chiefly by club shapes. It retains Gram's stain. On *serum* slender segmented bacilli showing a fair amount of metachromatism are seen. On *serum* discrete semi-transparent colonies occur, whitish in 24 hours, but taking on a yellowish colour in 48 to 60 hours, by which time many have coalesced into a thick layer. The growth afterwards becomes dry and powdery in appearance. On *gelatin* there is a good growth, white at first, later citron coloured. On *potato* there is a very slightly raised yellowish powdery growth. In *broth* a granular deposit is formed, but the medium remains clear. In *milk* a good growth takes place, but the medium remains unaltered. There is no acid, gas, or indol production. Non-pathogenic.

Diphtheroid 2. *Morphology.* Short pleomorphic bacillus showing segmentation of its protoplasm, metachromatic granules and early involution forms characterised chiefly by club shapes. Retains Gram's stain. On *serum* the bacilli resemble the "sheath form" of the diphtheria bacillus. On *serum* large, discrete, raised, spherical, opaque, grayish colonies are formed and the growth in some cases is confluent. On *agar* small, discrete, raised and transparent colonies are produced. After a few days' growth the colonies become faintly pink. On *gelatin* there is at first a good growth of discrete colonies which afterwards become confluent. The more superficial portions of the gelatin show a faint claret colour in about three days (the colonies remaining white or gray), which becomes deeper and tinges the whole of the gelatin in about a week. The colonies then acquire a faint rose pink tint. On *potato* a whitish shining growth occurs on the surface which becomes pinkish, and in three days the medium itself acquires a rose pink colour which becomes deeper. *Broth* becomes uniformly clouded, with a small amount of deposit at the bottom of the tube. A slight amount of acid is produced, but there is no gas or indol production. Non-pathogenic.

Diphtheroid 3. *Morphology.* Pleomorphic bacillus showing segmentation of the protoplasm and early club-shaped involution forms. On *serum* the majority of the bacilli stain badly. Some, however, stain well, show polar granules, and retain Gram's stain. On *serum* it forms a white, opaque, confluent growth, which appears as a film over the entire surface of the medium. On *agar* a good growth takes place in the form of raised, circular, white, opaque shining colonies, discrete at first, but tending afterwards to become confluent. On *gelatin* white or buff coloured very small colonies appear after several days growth. There is no growth

on *potato*. In *broth* growth occurs as small confluent masses which fall eventually to the bottom of the tube. There is no acid, gas, or indol formation. No macroscopic change takes place in milk. It is non-pathogenic.

Eyre concludes by saying that from his investigations "It follows that it is practically impossible to diagnose the presence of the true diphtheria bacillus in a milk sample by microscopical tests alone, but that the identity of the Klebs-Loeffler bacillus can only be established by careful consideration of its biological and pathogenic characters."

Summary of Chapter VIII.

Patients suffering from diphtheria transmit the disease to others, either through the direct deposition of infected material on to the mucous surfaces or hands of their attendants or friends, or through the agency of infected linen, toys, etc. The extent to which the disease may be spread by patients suffering from clinically typical diphtheria depends on the measures adopted to secure complete and efficient isolation, and may be very great in those cases in which the arrangements for isolation are defective. Persons suffering from unrecognised diphtheria are a far greater source of danger to the community than those who develop the disease in a typical form, and are frequently the cause of extensive outbreaks. Such atypical forms of diphtheria may include nasal catarrh, membranous rhinitis, atrophic rhinitis, tonsillitis, acute and subacute, otorrhoea, and apparently simple catarrh. Other persons acquire the bacilli but develop no symptoms, but are nevertheless capable of infecting others in the same way, and of giving rise to diphtheria. Children suffering from unrecognised diphtheria and healthy children infected with diphtheria bacilli spread the disease mainly by means of articles which pass from mouth to mouth, such as pencils, toys, and cups. Personal infection through these agents is by far the most important factor in the dissemination of diphtheria, and is the only one which plays an important part in most epidemics.

Infected milk has apparently been the cause of some widespread outbreaks.

All other possible means of transmission appear to play a comparatively unimportant part. A few undoubted instances of infection through animals and of accidental infection from cultures are recorded.

Very little bacteriological evidence can be produced to support the alleged instances of air-borne transmission, or infection through soil, dust, drains, sewer gas or decomposing refuse.

CHAPTER IX.

BACTERIOLOGICAL DIAGNOSIS.

Direct examination of cover-glass preparations. Swabs. Inoculation of culture media. Choice of media. Temperature and time of cultivation. Methods of making preparations for microscopical examination. Smears. Preparations from separate colonies. Staining of fixed preparations. Significance of polar bodies. The recognition of diphtheria bacilli under various conditions. Methods of obtaining pure cultures. The distinguishing cultural and pathogenic properties of the diphtheria bacillus. Method of recording the morphological appearances. Unusual diphtheritic lesions of the mouth. Diphtheria-like organisms found in the mouth. Diphtheritic lesions of the nose. Diphtheria-like bacilli found in the nose. Diphtheria of the eye and diphtheria-like bacilli found in the eye. Diphtheria of the ear and diphtheria-like bacilli found in the ear. Diphtheria of the skin and diphtheria-like bacilli found on the skin. Diphtheria of the female genital organs and diphtheria-like bacilli found on them. Diphtheria of the male genital organs and diphtheria-like bacilli found in the urethra. Diphtheria of the anus and rectum. Diphtheria and diphtheria-like bacilli in the urine.

Direct Examination of Cover-glass Preparations.

For the purpose of rapid diagnosis, and as an aid in the examination of cultures, the direct examination of smears made from pieces of membrane or material from the surface of swabs has been greatly used. These smears, made on cover-glasses and allowed to dry, are fixed by rapidly passing through the flame or by the application of absolute alcohol, and are subsequently stained by Loeffler's methylene blue, or by Neisser's method, or some modification of it, and mounted. The general arrangement of the diphtheria bacilli, and their staining properties under these conditions, have already been described (p. 124). By means of smears a correct diagnosis of the presence of diphtheria bacilli can frequently be made in clinical cases of diphtheria, but even in these cases the subsequent examinations of cultures show diphtheria

bacilli to be present in a considerable proportion of cases in which they had not been recognised in the smears. It follows, therefore, that very little trust can be placed on negative smear examinations, even in clinical cases, and none whatever in the case of convalescents, and contacts.

Again a positive diagnosis of the presence of diphtheria bacilli may not be confirmed by the culture test, since, whatever staining method may be used, other organisms are apt to be mistaken for diphtheria bacilli. Irregular, spindle-shaped bacilli and fragments of streptothrices, somewhat resembling true bacilli, are frequently met with in smears, and implicit reliance cannot be placed on Neisser's or other special stains since cocci and bacilli showing polar granules are of common occurrence.

The statistics of a carefully controlled set of experiments are quoted below (Mason, 1901):

Swab and serum culture both positive	37
Swab negative, serum culture positive	10
Swab doubtful, serum culture positive	7
Swab positive, serum culture negative	3
Swab and serum culture both negative	34
Swab doubtful, serum culture negative	6
Swab doubtful, serum culture no growth	2
	99

By culture tests 54 of these cases showed diphtheria bacilli, but only in 37 or 68 % was the swab diagnosis positive, and amongst the 45 cases negative by culture three (6·6 %) in smears showed organisms which were taken to be diphtheria bacilli, and eight (17·7 %) showed doubtful organisms. In this series including the doubtful cases, the smear diagnosis differed from the culture diagnosis in 28 %.

Hector (1905) examined 100 consecutive cases both by direct smears from the swabs and by cultures. In no case were diphtheria bacilli diagnosed in the smears and not subsequently found in the cultures. Cultures from 36 cases showed the presence of diphtheria bacilli, but they were only recorded as present in smears from 21 (58·5 %) of these.

Pugh (1905) using a special stain (p. 146) found diphtheria bacilli with definite polar bodies in smears made direct from the swab in 231 (57 %) out of 402 examinations. Cultures from all these cases were positive.

Beaton, Caijer, and Pakes (1901) and Cammidge (1901) believe that a trustworthy positive result can be obtained from the swab

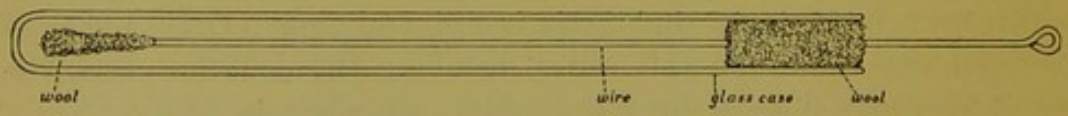
direct. Salus (1902) is satisfied if he finds diphtheria bacilli in one out of five smears from diphtheria cases.

Concetti (1900) advises the use of glass rods wrapped round with cotton wool impregnated with glycerinated glucose agar. One of these is rubbed on the throat, replaced in the tube, and placed in the incubator. After a few hours colonies develop and a smear is made from the rod and examined.

Smears are, however, of great use in differentiating Vincent's angina from true diphtheria, since the fusiform bacilli constantly associated with the former disease cannot be cultivated aerobically. *Bacillus fusiformis,* and the spirilla which frequently accompany it, can be readily recognised in smears, and attention directed to the probable cause of the disease, before cultures can be examined (see Chapter X).

Bacteriological Diagnoses by Means of Cultures.

Swabs. Swabs are now in general use for obtaining the material with which the culture tubes are inoculated. The swab consists of a small quantity of cotton wool wrapped round a stout wire, the end of which is either roughened or slightly flattened. The wires are placed in glass or metal cases, so that the whole may be sterilised. Sterilisation is generally accomplished by heating to 160° C. in a hot air steriliser for two hours. A convenient and cheap form of swab is illustrated below:



Platinum loops and various other devices have been made use of instead of swabs.

Swabbing. In taking a swab from the mouth care must be exercised not to touch the surface of the tongue or other parts, either when introducing or removing the swab. It should be rubbed on the surface of the membrane or exudate, or, if neither of these is present, over the diseased area, and immediately replaced in its case. Pus or discharge from the nostrils can also be collected on swabs.

In the case of contacts the swabs should be taken from any unhealthy areas that may be seen and also from the surfaces of both

tonsils. Where exudation is seen from any of the crypts an attempt should be made to inoculate the swab with some of this material.

Occasionally it is possible to express purulent material from the crypts of the tonsil, when very little can be seen on the surface, by pressing the tonsil inwards with the fingers below the angle of the jaw and rubbing the surface with the swab.

Swabs should not be taken shortly after the application of antiseptics, as the growth of the bacilli on the culture media is thereby likely to be hindered or entirely inhibited.

Under certain circumstances it may be impossible to sow the swab on culture media for several hours. Under these conditions it is advisable to moisten the surface of the swab with sterile water or broth before it is taken.

Inoculation of Culture Media.

For the purposes of routine examinations swabs are usually sown on the sloped surfaces of serum tubes, each swab on a single tube. The general custom is to thoroughly rub the swab over the surface of the medium in such a manner that all parts of the swab come into contact with the surface. In certain cases where there is likely to be a very copious growth the writer has found that a very satisfactory method is to rub the swab thoroughly over one side of the slope and pass it lightly over the other. The result is that at least on one part of the slope more or less separate colonies can usually be found. For special investigations the swabs may be rubbed over the surfaces of several tubes in succession, so that under any circumstances discrete colonies may be obtained. For this purpose plate cultures may also be employed, or small pieces of membrane or material from the surface of a swab may be made use of to inoculate sterile salt solution or broth tubes, and cultures be subsequently made from these.

Choice of Media.

Loeffler's medium made from horse or ox serum has been most extensively used. Kanthack's serum has also been employed by several observers, and Cobbett's modification of Lorrain Smith's serum medium was used by Cobbett in his investigations at Cambridge (see p. 150). The writer has also used this medium in the examination of several thousand cultures.

Comparative tests between Cobbett's medium and Loeffler's have convinced the writer that diphtheria and Hofmann's bacilli grow equally well on both media, and that consequently owing to its transparency the alkaline serum is the most convenient for diagnostic work, since the separate colonies are more easily seen and their characters recognised, and it is simpler to obtain specimens from single colonies for microscopic preparations or the making of subcultures.

So far as the writer's experience goes media made from human exudates are unsatisfactory, as Hofmann's bacillus is apt to resemble the diphtheria bacillus more closely than when media made from ox serum are used.

Temperature of Growth.

The cultures are generally incubated at 37° C., but various temperatures have been advocated between 35·5° C. and 37° C. Those who advocate the lower temperatures think that diphtheria bacilli alone develop polar bodies within a given time (18 to 20 hours), and may therefore be diagnosed by their presence (see p. 142).

Time of Cultivation.

The great majority of observers examine their cultures after 12—18 hours' growth, and most of them do not re-examine them after further growth. As has already been pointed out diphtheria and Hofmann's bacilli generally develop recognisable colonies in this time, whereas the majority of organisms found in the throat do not show visible growth on serum after this period of incubation. Certain cocci and bacilli do, however, form colonies within this period.

Methods of making Preparations for Microscopical Examination.

(1) *Smears made after a few hours' growth.* Ohlmacher (4. v. 1895) has recommended scraping the surface of the culture medium with a platinum loop after four hours' incubation at 37° C. He states that under these conditions "no trouble will be experienced in finding plenty of organisms upon which to base a satisfactory diagnosis."

The smear on the cover-glass must occupy a very small area in order that the organisms may not be too widely scattered and consequently missed. This method was advocated purely for the purpose of obtaining a very rapid diagnosis by culture.

(2) *Smears made from the surface of the culture after* 12—18 *hours' growth.* Many observers prepare smears on the cover-glasses by scraping material from the surface of the cultures. This method of obtaining preparations regardless of the separate colonies has been described earlier (p. 213). For the purpose of scientific research the results obtained from such preparations are obviously useless, since nothing but the morphology of bacilli inextricably mixed from various colonies can be determined.

It is impossible to state whether the various types found came from separate colonies or were present in single colonies. Further, if the whole surface of the tube has been scraped, it becomes hopeless to obtain pure cultures and test the accuracy of the diagnosis, and a second examination of the culture after further growth cannot be made. It necessarily follows that those who habitually employ this method give their diagnosis on certain fixed conceptions of diphtheria and other bacilli. They probably neither prove nor disprove the value of their diagnoses, and the errors are likely to be multiplied and perpetuated.

An example of the effects of such proceedings may be taken from Pl. XII, fig. 5. In this culture some of the larger colonies have been produced by Hofmann's bacilli and others by cocci, while the smaller colonies are those of the *B. coryzae segmentosus.* A smear from such a culture would probably lead to a diagnosis of diphtheria, since the latter organism (Pl. XIV, fig. 1) bears a striking resemblance to the segmented diphtheria bacillus. It would also be stated that Hofmann's bacilli occurred in conjunction with diphtheria bacilli. In other cases bacilli differing from diphtheria bacilli in every characteristic except morphology must be diagnosed as diphtheria bacilli. A more careful method at once obviates these sources of error. Even for the most superficial of routine examinations there is nothing to recommend the use of such smears except the saving of labour and time at the expense of accuracy.

(3) *Preparations made from separate colonies.* For the purpose of the routine diagnosis of diphtheria, preparations from separate colonies can be made with nearly as much ease as smear preparations, and the results are infinitely more satisfactory and reliable, and, for the purpose of obtaining statistics of scientific value or the examination of contacts or convalescents, are the only ones which are trustworthy. Several preparations from different colonies can be made on one cover-glass, and under these conditions can be examined faster than smear preparations. In the latter it is necessary to thoroughly examine the

whole smear in order to determine the absence of any given organism; in the former an examination of any part of a preparation from a single colony suffices. Preparations from single colonies are made in the following manner: the top of a colony is touched with a sterile needle, which is then dipped in a capsule of water and drawn two or three times along the cover-glass so as to produce a streak. The same process is repeated with different colonies until several lines (5 or 6), composed of micro-organisms from separate colonies, are made in parallel series on the cover-glass.

It might be thought that the water would become so contaminated that the preparations from the various colonies would eventually show several kinds of organisms. In practice, however, this is not the case. For greater accuracy minute drops of water may be placed in a row on the cover-glass and the inoculated point of the needle dipped in one of these before the streak from it is made. In all cases streaks should first be made from diphtheria-like colonies, and then from colonies more or less resembling them. Streaks from colonies widely differing from those of the diphtheria bacillus are made still later. If the examination of the diphtheria-like colonies does not reveal diphtheria bacilli several typical colonies from various parts of the tube should be examined. In all cases it is desirable as far as possible to examine all dissimilar colonies; since many of the organisms morphologically resembling diphtheria bacilli produce colonies totally different in appearance.

It occasionally happens that in crowded cultures the colonies are so small and closely aggregated that separate colonies cannot be examined. In these cases streaks may be made in the manner described from groups of similar colonies.

This method has numerous advantages over the smear method. Firstly, the colonies may be examined and the bacilli producing the various types recognised. In this way the writer has on several occasions been able to give a satisfactory diagnosis on organisms, which, from their microscopic appearance alone, might have caused trouble, but which formed colonies entirely different from those of the diphtheria bacilli. Further, the various types of bacilli present in single colonies can be determined, a matter of some interest in view of the assertion that several types of diphtheria bacilli may be met with in single colonies. The staining reactions of the various organisms forming different colonies can be observed, which is impossible in smear preparations. Colonies of doubtful bacilli can be easily picked out for making subcultures and proving their identity.

This method allows also of the culture being further cultivated and the separate colonies being again examined after a longer period of growth.

Although in typical cases of diphtheria little advantage is likely to be gained by the latter proceeding except when slow growing varieties of the diphtheria bacillus are present (p. 246), or the previous application of antiseptics has hindered development, yet in the case of contacts and convalescents, as far as the writer's experience goes, the second examination frequently reveals isolated colonies of the diphtheria bacillus and also of Hofmann's bacillus, which had either been overlooked owing to their small size, or had not developed at the previous examination. This is especially the case when the patients are being constantly treated with antiseptics in order to hasten the disappearance of the bacilli. Such bacilli would be in the majority of cases entirely overlooked in general smears since a single minute colony contains but few bacilli, and the probability of not finding them in the smear, or of their not being included, is very great. During the last three years the writer has, as a routine practice, made a second examination of all cultures in which diphtheria bacilli were not originally found, and has discovered diphtheria bacilli in the second examination in 144 (5·8%) out of 2454 cultures from 285 contacts and convalescents. These second examinations also frequently revealed the presence of isolated colonies of Hofmann's bacillus. Considerable importance ought therefore to be attached to second examinations, since no observations on the classes of persons just mentioned can be considered complete unless cultures, which at first show no diphtheria bacilli, are re-examined after a second period of incubation. Second examinations also frequently reveal the presence of Hofmann's bacilli in many healthy throats, in which their numbers are very small. Although absolutely accurate statistics of the number of times Hofmann's bacillus was discovered in the second and not in the first examination cannot be obtained from the writer's records, yet the unusually large percentage of normal persons recorded in Table D, p. 211, as harbouring these organisms is due in some measure to these secondary examinations. It was seldom found that they were missed on the first occasion, except in cases in which only one or two colonies were present amongst a copious growth of other organisms.

Staining of Fixed Preparations.

Loeffler's method is the one which has been most generally employed for staining the fixed preparations in whatever manner they have been made. This method when properly applied has been stated by many observers to give all the information which can be derived from the accessory methods of Neisser and others. The bodies of the bacilli show the characteristic irregularities in the protoplasm extremely well, and the metachromatic staining, in those which possess it, is well exhibited. Certain observers, therefore, state that for routine diagnostic purposes no other methods are necessary.

On the other hand, there are many workers who consider that Neisser's stain, or one of the other differential stains for the polar granules, is of great assistance, and almost invariably stain a second preparation from suspicious cultures in order to determine whether these granules are present or not.

Both these methods can be combined and much time saved by the method devised by *Cobbett*. This process consists of making on a cover-glass several parallel streaks from different colonies. The cover-glass is allowed to dry and then dropped film side down on a glass slide on which has been placed a drop of dilute Loeffler's methylene blue (1 : 5). The specimen is almost immediately firmly pressed down on filter paper with the cover-glass downwards, with the result that the excess of stain is forced from under the cover-glass and absorbed by the filter paper.

Immersion oil is then placed on the cover-glass and the preparation examined, mounted in a small quantity of the staining fluid. If the films are properly prepared[1] no bubbles are found under the cover-glass, and the organisms take up the stain and appear to lie in a clear fluid (Cobbett and Phillips, XII., 1896, p. 197). By this method the more concentrated portions of the protoplasm of the diphtheria bacilli are stained dark blue, the other portions light blue and the polar bodies are usually indicated.

Hofmann's bacilli stain darkly except for the median light band, and amongst these well stained specimens numerous almost unstained "shadows" are to be seen.

This method has very considerable advantages over the method of

[1] It has been stated that the cover-glass is apt to stick to the objective. This does not occur if large cover-glasses are used, and the excess of stain is removed by firm pressure.

staining by Loeffler's blue and mounting in Canada balsam. In the first place much time is saved, the bacilli are not overstained nor are they liable to be distorted during manipulations or by the action of balsam. Comparative experiments have convinced the writer that in balsam the diphtheria bacilli appear smaller and shrunken, and are much less easily distinguishable from other organisms. Finally this procedure allows of 5 % acetic acid being run under the cover-glass as recommended by Cobbett (IX. 1901). A drop of the acid placed near the edge of the cover-glass generally runs quickly under the latter, and, if it does not do so, the withdrawing of a little fluid by placing a piece of filter paper at the opposite edge generally causes the acid to run in. If the preparation be watched during this process a wave of blue is seen to pass over it, and the bacilli are left almost decolorised except the polar bodies which stand out as dark black or blue dots. A single group of doubtful organisms can thus be readily examined for the presence of polar bodies without removing the eye from the microscope, a matter of great importance when the organisms are few, and it is doubtful whether they can again be obtained for making a preparation by Neisser's method.

Comparative experiments with this method and that described by Neisser show the results to be identical. The writer has used the method just described for the examination of several thousand cultures, and has found it extremely satisfactory in all respects.

Significance of Polar Bodies.

Although the presence of polar bodies is an aid to diagnosis yet, as has already been stated (p. 143), many diphtheria bacilli show none, and even when well developed their presence does not indicate that the organisms are virulent (p. 197). Further, several other species of bacilli resembling diphtheria bacilli also develop well-marked polar bodies within the usual limits of time in which cultures are examined. Hofmann's bacillus as a general rule in young cultures has no polar bodies, but in a few instances inconspicuous polar bodies few in number may be seen. In older cultures of this organism polar bodies are not infrequently seen. The polar bodies in these cases are generally, however, not so distinct, nor so dark, nor as uniform in size as those of the diphtheria bacillus. This fact is of importance when an examination of a culture 36 or more hours old is being made.

The Recognition of Diphtheria Bacilli.

The purpose for which the examination is made to some extent determines the time which can be devoted by the observer to the recognition of the bacilli. Bacteriological examinations may be undertaken for the following purposes :—(1) routine examinations for the diagnosis of diphtheria; (2) for the discovery of infected contacts; (3) for the release of convalescents and infected contacts from quarantine; (4) for the diagnosis of doubtful diphtheritic lesions in other situations than the throat and nose; (5) for proving the relationship of diphtheria and diphtheria-like bacilli to various morbid processes; (6) for obtaining statistics on the distribution of diphtheria bacilli amongst the normal population.

(1) In routine examinations undertaken for the purpose of giving opinions on the nature of cases clinically resembling diphtheria a diagnosis is expected in the majority of instances within 12 or 18 hours. The morphology and staining reactions together with the characters of the young colonies are the only features on which an opinion as to the nature of the organisms can be formed. The observer has therefore to rely on the results of his previous experience, since to be of any practical value the opinion of the bacteriologist cannot await the preparation of pure cultures and the injection of animals. Of the nature of bacilli which exhibit the typical characters of diphtheria bacilli there can be little doubt, but organisms will occasionally be met with which differ from the usual types in some particulars, and which have not been previously encountered by the observer. Under these conditions, wherever possible, a definite answer should be delayed, and the organisms subcultured so that their characters may be noted when not influenced by the presence of contaminating organisms. A longer delay will seldom be possible, and a diagnosis must be given on the data which have been obtained. It remains, however, for the bacteriologist to prove for his own benefit whether the opinion given was correct, by obtaining pure cultures and testing the principal cultural characters of the organism as well as its effect on animals. Unless such methods are adopted errors of judgment can only be perpetuated, and the opinion of the observer, however large his experience, will be of no value in doubtful cases. In the case of certain institutions and especially of private schools an opinion should be delayed when doubtful organisms are found in a primary case of supposed diphtheria. A

positive diagnosis of diphtheria not infrequently involves the owners in considerable pecuniary loss, and the patient can generally be isolated until certain characters of the bacilli have been tested. Unless the cultures are very greatly contaminated a pure culture can generally be obtained and its action on glucose broth tested within three or four days.

In the writer's experience bacilli which to some extent resemble diphtheria bacilli, but do not form acid in glucose broth, are not pathogenic to guinea pigs, and are therefore probably incapable of causing diphtheria in man. This experience is in complete agreement with that of Cobbett and many other observers. On the other hand bacilli which do form acid and grow in the typical manner of diphtheria bacilli in broth, and which are derived from persons suffering from lesions in any way resembling diphtheria, usually prove to be virulent diphtheria bacilli. In conclusion it may be said without hesitation "that when once one has become fully acquainted with the range of its variation it is fairly easy to recognise the diphtheria bacillus and distinguish it from all others," but "the eye cannot be sufficiently trained for this purpose unless the observer frequently tests the opinions he forms on morphological grounds by isolating his cultures, and testing them in various ways, including the injection of animals" (Cobbett, IV., 1901, p. 236).

(2) The examination of contacts should be carried out with the same precautions, and in their case it is almost always possible to delay a definite opinion, until at least the action of pure cultures of doubtful bacilli on glucose media has been determined. Here again much inconvenience and even pecuniary loss may be caused by hasty opinions, which are subsequently proved to be erroneous. The value of a further period of incubation for negative cultures and subsequent second examinations in these cases has already been pointed out.

(3) The examination of convalescent cases of diphtheria and of infected contacts for the purpose of deciding whether the diphtheria bacilli have disappeared, or not, is during the earlier stages usually an easy matter, as the organisms are frequently abundant. In the later stages, however, when the bacilli are beginning to disappear a negative verdict should not be given until the culture has been thoroughly examined after a further period of incubation, and doubtful bacilli, as in all other cases, should be carefully tested. As long ago as 1895 Ohlmacher (2. III. 95) pointed out that diphtheria bacilli may sometimes be missed in cultures from convalescents unless they are incubated for longer than the usual time.

(4) The examinations of discharges from the female genital organs, ears, ulcers, abscesses, etc., and of skin lesions[1], and also of milk and various other materials, for the presence of diphtheria bacilli are rendered difficult by the fact that in all these situations innocuous diphtheria-like bacilli are frequently present, which are in no way related to the disease. In such examinations, therefore, no final opinion should be given until the identity of the organisms has been thoroughly proved, however closely they resemble diphtheria bacilli.

(5) Mention must also be made of a class of investigation which aims at proving the association of diphtheria or diphtheria-like bacilli with certain morbid processes, not generally recognised as being related to diphtheria. It would seem scarcely necessary to point out that the bacilli should be tested in every possible way, including the effect of animal inoculations. Nevertheless many hasty generalisations, which if adopted would have the most far reaching consequences, have been based on morphology alone. To those who have any acquaintance with bacteriology the worthless nature of such observations is obvious, but they are apt to mislead a large number of persons who have no practical acquaintance with the subject, but are willing to place reliance on the conclusions of bacteriologists.

(6) Another class of observations to which attention has to be directed is that which deals with the presence or absence of diphtheria bacilli in the mouths of perfectly healthy persons, who have not recently had an opportunity of acquiring diphtheria bacilli by contact. It has already been pointed out (p. 189) that, if these observations are to form a basis for general arguments, all possible recent contacts must be excluded. Further, the animal inoculation test must be applied to all organisms which the observer considers to be diphtheria bacilli, since it has been shown that almost all the so-called diphtheria bacilli which have been found amongst these persons have been devoid of virulence. Whether the non-virulent diphtheria bacillus will ultimately be proved a distinct species from the virulent, as some observers think, or not, there is some evidence to show that the former is not capable of giving rise to diphtheria in man. Any arguments, based on the prevalence of diphtheria bacilli amongst the normal population and the transmission of the disease by this means, ought to take these facts into account. Many of the observers have, however, not tested the pathogenic properties of the bacilli they have found, or only have tested a small percentage of them,

[1] The characters of some of the organisms found in these situations are given in the following pages.

but in many instances they have assumed that all morphologically typical diphtheria bacilli must be potential carriers of the disease.

One of the latest and most extended investigations which have been carried out on the occurrence of diphtheria bacilli in well-persons is that of the Massachusetts Association of Boards of Health (1902). The several collaborators were requested to detail the various bacilli according to Wesbrook's types, and also to state in each case whether or not a positive diagnosis of the presence of diphtheria bacilli had been made. The different collaborators held divergent views as to which of the various types should be considered as true diphtheria bacilli, and in this investigation only a small proportion was tested for virulence. This report gives a good example of the unsatisfactory confusion to which a lack of thorough testing can lead.

In Providence (*Report*, footnote, p. 24), on the basis of the Committee's belief that A, C, and D of Wesbrook's types should be considered chiefly, or solely, important, there would be ·43 % of positives (*i.e.*, cultures in which diphtheria bacilli were present). If all granular, or barred forms, but not the solid forms, be included, as Prof. Gorham of Providence states, there would be 3 % of positives. If all be included there would be 25 %. The number actually reported positive (*i.e.*, diphtheria bacilli present) makes about 9 %.

In Washington the positives formed ·9 % on the Committee's standard, but 22 % were reported positive. In Boston on the Committee's standard 3·02 % were positive, but only 1 % was so reported.

The whole question of the recognition of diphtheria bacilli may be summed up in a few words.

In the practical diagnosis of diphtheria it must be assumed that organisms morphologically resembling diphtheria bacilli are true diphtheria bacilli, for the opinion of the bacteriologist to be of any practical value cannot await the preparation of pure cultures and the injection of animals; but if far reaching deductions are to be drawn from the observations the testing of the organisms found is essential, especially if the deductions are to be made the bases on which epidemics of diphtheria are to be combated by bacteriological means.

Apparently in consequence of omitting to verify their diagnoses by isolating and testing doubtful organisms, very divergent views are held even at the present day by various observers as to which of the various types of organisms should be considered dangerous. This unsatisfactory confusion renders many of the investigations untrustworthy, and can only lead to the discredit of the bacteriological diagnosis of diphtheria.

The diphtheroid organisms met with in cultures are either diphtheria bacilli (virulent or non-virulent) or they are not diphtheria bacilli. By means of cultural tests and animal experiments it is generally easy to distinguish between these groups; and even on morphology alone it is usually not difficult to give an accurate opinion, provided that the observer has systematically identified the organisms he has previously met with. It is therefore the duty of every bacteriologist who has to deal with this disease to make himself acquainted with the range in variation of the diphtheria bacillus and the organisms which resemble it, and to verify his diagnosis by every means in his power. By the adoption of these means alone can the opinions, which he gives on the examinations of his cultures, be of any value. It cannot be too often repeated that the examination merely of large numbers without proper controlling tests and without a knowledge of the origin of the cultures cannot be of service in the formation of trustworthy opinions.

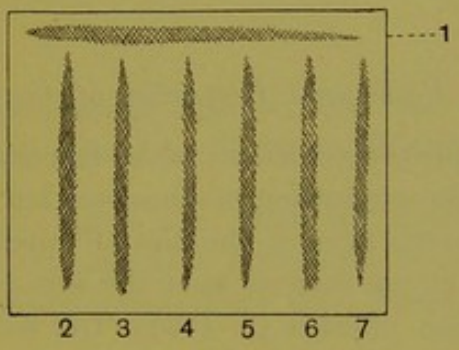
Methods of obtaining Pure Cultures.

From the majority of original cultures it is easy to obtain pure cultures in one or two generations. The simplest method is to touch a suspected colony with the point of a sterilised platinum needle and immediately inoculate a tube of sterile salt solution (·7 %). In order to ascertain whether the type of bacillus, which it is desired to investigate has been sown in the salt solution, a smear is made from the needle on a cover-glass immediately after the former has been removed from the salt solution and before it has been sterilised. A minute drop from the salt solution tube is inoculated by means of a sterile platinum loop onto the surface of another serum tube. After incubation well separated colonies develop. Should the culture thus obtained be found by microscopic examination to contain colonies of other organisms besides those which are under investigation, the same procedure is repeated and a pure culture is obtained.

If the colonies on the original culture are very minute and the culture is not greatly crowded with contaminating colonies, further incubation adds to the growth of the separate colonies, and a pure culture can be obtained with greater ease. From very crowded cultures with minute colonies the first subculture is certain to be impure, but a pure culture can generally be obtained in two generations.

More difficulty is experienced when only one or two colonies of

doubtful organisms are present amongst many other colonies which resemble them. In this case several salt solution tubes may be inoculated from difficult colonies, and numbered. Before sterilising the needle on each occasion a streak is made on a cover-glass. The streak corresponding to the first tube should run along one side of the cover-glass and the other streaks should be made in order at right angles to it, beginning on the left. There are then on one cover-glass several streaks from different colonies, corresponding to the salt solution tubes which have been sown from these colonies, arranged as shown in the following figure:



If the cover-glass is now stained and mounted it can readily be seen by noting the number of the streak, which shows the desired organisms, which salt solution tube must be used for subcultivation.

Various other methods, such as plating on agar, etc., may also be used for obtaining pure cultures.

The Distinguishing Cultural Characters of the Diphtheria Bacillus.

The principal cultural character, which distinguishes the diphtheria from the Hofmann's bacillus, is the formation of acid by the former in media containing glucose as opposed to the alkaline reaction produced by the latter. Abundant acid production also distinguishes the diphtheria from the xerosis bacillus, which produces, according to some observers, a slight amount of acid, according to others, none.

Certain other diphtheroid organisms also produce acid, but differ from the diphtheria bacillus in other respects, as in the appearance of their colonies and their rate of growth on various media.

Both the diphtheria and the Hofmann's bacillus produce an invisible or almost invisible growth on potato, while many of the diphtheria-like organisms produce abundant, and frequently coloured, growths.

The peculiar granular deposit together with the lack of clouding in broth cultures distinguishes the diphtheria bacillus from the Hofmann's bacillus, which produces more growth and some clouding, and from some of the other diphtheroid bacilli which produce very different growths. Neither the diphtheria bacillus nor the Hofmann's bacillus produces pigmented colonies on any media except serum, and even on this medium a yellowish coloration of the colonies is the only evidence of pigment production. Some diphtheria-like organisms liquefy gelatin.

So far as the writer's experience goes all organisms which produce an alkaline reaction to litmus in glucose broth are found to be non-pathogenic.

The Distinguishing Pathogenic Properties of the Diphtheria Bacillus.

The virulent diphtheria bacillus when inoculated in pure culture under the skin of the guinea-pig produces certain well-marked lesions, namely a small necrotic area at the site of injection, gelatinous subcutaneous oedema, intense congestion of the suprarenals and frequently clear yellow pleuritic effusion (p. 173). The simultaneous injection of antitoxin together with the culture protects the animal from the effects of the bacillus.

Less virulent diphtheria bacilli or smaller doses produce only oedema from which the animals recover, and these effects are also counteracted by antitoxin.

Experimental animals which recover either with or without the aid of antitoxin frequently suffer from paralysis.

Since some of the diphtheria-like organisms kill the inoculated animals by a septicaemic process (antitoxin either producing no effect or hastening death), autopsies on the experimental animals ought never to be omitted.

The Methods of recording the Morphological Appearances of Diphtheria Bacilli.

For recording the morphological appearances of diphtheroid bacilli met with in cultures Wesbrook's classification may be conveniently used. It must, however, be borne in mind that by this means a general idea of the morphological appearance of an organism alone is given, but no proof that it bears any relationship to the diphtheria bacillus. In most cases it is found that the majority of organisms

composing a colony are of one type, but others belonging to one of the other types also occur. In recording the morphology of a pure culture or colony the general appearance conveyed to the eye is usually of more importance than a minute analysis of the slight variations from the general type, which may be found by diligent search. The method of growth of diphtheria and allied bacilli, and the different ages of the individuals comprising the colony, make it certain that a variety of individuals will be found. Nevertheless any pure culture has a characteristic general appearance, and most of the individuals belong to one type. Consequently in describing a culture it is the general character which it is important to convey.

Uncommon Diphtheritic Lesions, and the Diphtheria-like Bacilli found in Various Situations.

In the following pages are given a number of instances of diphtheritic lesions in unusual situations, and the characters of diphtheria-like organisms which have been found in these situations.

The mouth.

Diphtheritic lesions of the mouth are usually either confined to the neighbourhood of the tonsils, or are most marked there, and spread to other situations. Some of the more remarkable examples of diphtheritic lesions affecting other portions of the buccal cavity are quoted below:

Stomatitis. Councilman, Mallory, and Pearce (1900) observed general stomatitis due to diphtheria bacilli in five cases. *Tongue.* Membrane on the tongue due to virulent diphtheria bacilli has been noted by Wharton (1895). Trevelyan (1900), Thiercelin (1890), and Salmon (1904) have also observed membranous lesions on the tongue associated with diphtheria bacilli. In Salmon's case membranes were also present on the lips, palate, cheek, vulva, vagina, and rectum. Animal inoculations do not appear to have been made in these cases. *Lips.* Trevelyan (1900) recounts a case of diphtheria of the inner surfaces of the upper and lower lips. Long diphtheria bacilli were found, but their virulence was not determined. Flexner and Pease (1895) quote two remarkable examples of diphtheria of the lips and gums first noticed at autopsies. "The appearances of the membrane in

one and the exudate in the other case were in no way typical of diphtheria, and the cases obtain an additional interest from the fact that during life the patients presented no symptoms referable to the presence of the Loeffler's bacillus. No other forms of diphtheria existed in the body so far as could be determined." In both cases the organisms were morphologically and culturally typical, but only caused a well-marked local reaction in guinea-pigs.

In the case of a patient suffering from diphtheria, who developed *herpes labialis* on the 9th day, Tez (1895) found virulent diphtheria bacilli in the vesicles. *Cancrum oris.* Several cases of cancrum oris associated with the presence of the diphtheria bacillus have been described. Freymouth and Petruschky (1898) observed two cases, Councilman, Mallory and Pearce (1900) two cases and Walsh (1903) eight cases. The first mentioned observers isolated the bacilli and found them to be in each case non-pathogenic for guinea-pigs in doses of 2 c.c.

PLATE XIV.

Fig. 1. Photograph of a smear preparation stained with Loeffler's methylene blue of *B. coryzae segmentosus*, grown on serum for 18 hours at 37° C. × 1000. (From Gordon, 1903, Plate VIII, fig. 9.) (See p. 361.)

Fig. 2. Photograph of a smear preparation stained with Loeffler's methylene blue of a chromogenic organism from the eye, grown on serum for 18 hours at 37° C. × 1000. (From Gordon, 1903, Plate VIII, fig. 7.) (See organism No. 22, p. 366.)

Fig. 3. Photograph of a smear preparation stained with carbol-fuchsin and Neisser's method of a streptothrix obtained from the throat of a diphtheria patient. One day agar culture grown at 37° C. × 1000. (From Gordon, 1903, Plate XI, fig. 17) (p. 357).

Fig. 4. Fusiform bacilli and spirilla in a smear preparation stained by Giemsa's method made directly onto a cover-glass from a case of Vincent's angina. Drawn with the aid of a camera lucida (Orig.). Zeiss $\frac{1}{12}$, Oc. 4.

Fig. 5. Photograph of a smear preparation stained with carbol-fuchsin of a pure culture of the fusiform bacillus grown five days anaerobically in dextrose-free broth. × 1200. (From Weaver and Tunnicliff, 1905, Plate XVI, fig. 1.)

Fig. 6. Photograph of a smear preparation stained with carbol-fuchsin of a pure culture of the fusiform bacillus grown for three days anaerobically on an ascites agar slant. × 1000. (From Weaver and Tunnicliff, 1905, Plate XVI, fig. 2.)

Diphtheria-like organisms from the throat.

A considerable number of organisms have been isolated from the throat which, although they resemble the diphtheria bacillus in

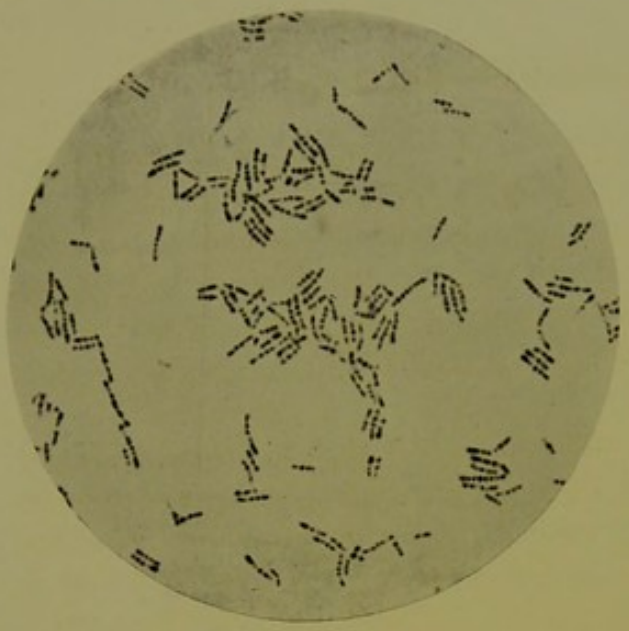


Fig. 1.

Fig. 2.

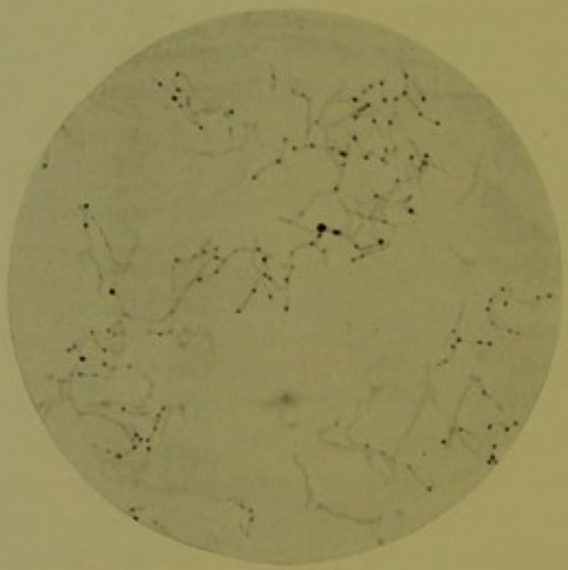


Fig. 3.

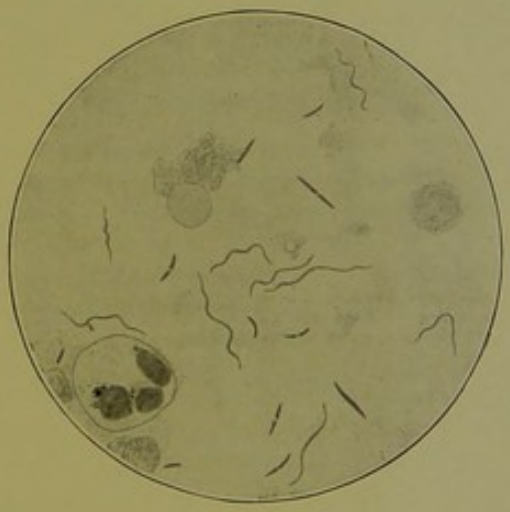


Fig. 4.

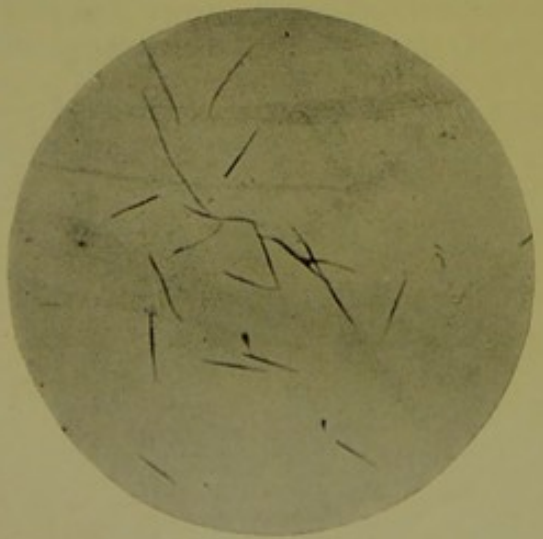


Fig. 5.

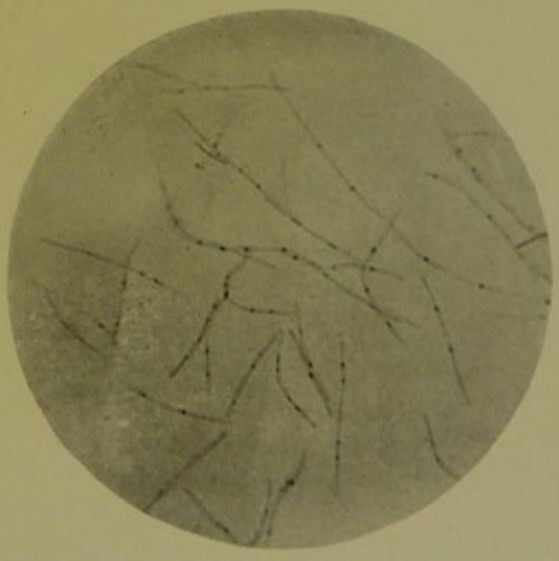


Fig. 6.

morphology, differ from it in certain of their characters. The true diphtheria bacillus is characterised by its:

(1) Macroscopic growth on *serum* and morphology, (2) power of producing acid in glucose broth, (3) invisible growth on potato, and (4) the lesions produced in inoculated guinea-pigs.

The diphtheroid organisms are here classified according to the number of points in which they differ from the diphtheria bacillus.

A. *Microorganisms resembling the diphtheria bacillus in all the main characters except virulence.*

Under this heading might be included the non-virulent diphtheria bacillus.

1.[1] *Bacillus maculatus.* (Plate XV, fig. 5.)

Origin. One colony found in a culture from the throat of a possible contact.

On serum in 24 hours the colonies are opaque white, but otherwise resemble those of the diphtheria bacillus. The organisms are longer and broader than diphtheria bacilli, but some short forms occur. The sides in some have slight bulgings at intervals. Numerous darkly stained segments cross the bacillus transversely in most of the organisms, but in a few there are oval segments. In some bacilli, especially in the later subcultures, long unstained intervals are seen. The organisms are non-motile, and retain Gram's stain very deeply. Each bacillus shows numerous polar bodies by Neisser's method; some of these are large and round, others elongated transversely across the bacillus, whilst others are very minute. These minute polar bodies are often very densely aggregated. The name indicates the remarkable spotted appearance seen when the organisms are stained by Neisser's method.

In the first culture the organisms lay in tangled masses of 10—50 individuals. On agar in 24 hours minute, round, transparent colonies are formed which subsequently grow very slowly. The organisms are about one-third the length of those found on serum cultures and of various shapes, from oval to bloated pear-shaped bodies. The polar bodies are few. On the surface of agar stab cultures an almost transparent film is formed, but in the depth medium-sized yellowish colonies grow. On gelatin after 10 days' growth the colonies are so minute as to be scarcely visible with a lens. Exceedingly minute colonies also form in the depth of gelatin stab cultures. On potato there is no visible growth. Broth remains clear, but a few large, discrete, yellow granules (0·5 cm. in diameter) are seen after 48 hours. The reaction of glucose broth becomes very faintly acid. Milk remains unchanged and no indol is formed. The organisms are non-pathogenic when injected either subcutaneously or intraperitoneally into guinea-pigs.

This organism differs from the diphtheria bacillus slightly in its morphology, its growth on gelatin, and in the very large size of the granules formed in broth, and the degree of acid formation in glucose broth.

[1] For the sake of reference the organisms described in the following pages are consecutively numbered.

B. *Organisms mainly differing from the diphtheria bacillus in their macroscopic growth on serum and in lack of virulence.*

Gordon (1903, p. 429) describes two species of bacilli of this kind.

2. After 18 hours' incubation a good growth was obtained on *serum*, but so coherent that to obtain a microscopical view of the individuals it was necessary to separate the growth by crushing between cover-glasses. Morphologically the bacilli are short and stout and resemble Hofmann's bacillus, retain Gram's stain and have well developed polar bodies at each end. On *agar* dry, white, circular, raised coherent colonies are formed. On *gelatin* there is no growth. *Broth* remains clear with coherent masses at the bottom. *Glucose broth* becomes acid. *Milk* is not changed. The organism is non-pathogenic.

3. After 18 hours' growth on *serum*, gray sharply outlined coherent colonies are formed. The organisms are curved and have swollen ends and occasional branching forms are encountered. There is however much variation in size and shape, some of the organisms being oval and some resembling mycelial threads. Loeffler's methylene blue stains them irregularly and they retain Gram's stain, but show no polar bodies by Neisser's method. There is no growth on *gelatin*. In *broth* coherent masses form at the bottom and *glucose broth* becomes acid. *Milk* becomes acid and by the 9th day is firmly clotted. Non-pathogenic.

C. *Microorganisms which resemble the diphtheria bacillus in all their main characters except virulence, but liquefy gelatin and cause clotting in milk.*

4. Gordon (1903, p. 431, No. 10).

Origin. Obtained from four patients suffering from diphtheria.

On *serum* after 18 hours moist raised gray colonies are produced. The organisms show great variations in size and shape, varying from oval to pear-shaped. Some very closely resemble diphtheria bacilli. Most stain evenly and deeply, but some show unstained portions and granules. Polar bodies are seen in many when stained by Neisser's method. They retain Gram's stain. On *agar* round, granular, raised colonies are formed which adhere to the medium and the organisms mostly resemble cocci, but some diphtheria-like forms occur. *Glucose broth* becomes acid. On *gelatin* there is a slight raised growth, and after 10 days' growth the medium begins to be liquefied. *Milk* becomes acid and on the 6th day is firmly clotted. Non-pathogenic.

D. *Microorganisms mainly differing from the diphtheria bacillus in their growth on potato and in their lack of virulence.*

5. *Bacillus diphtheroides citreus.* (Plate XV, fig. 3.) Graham-Smith (1904).

Origin. Obtained from the throats of five healthy children, two attending infected, and three non-infected schools.

On *serum* in 24 hours the colonies closely resemble those of the diphtheria bacillus, but are rather larger and more opaque. After three days' growth they become slightly yellowish. The organisms are fairly long and stain darkly, in

shape resembling Hofmann's bacilli, but with little trace of a median band. The majority are slightly curved, and show small terminal polar bodies, though in some of the larger forms they are very large and distinct. They are non-motile, and retain Gram's stain. In 48 hours' subcultures a number of long forms with three or four well-marked segments and distinct polar bodies occur. On *agar* in 24 hours large, white, round, smooth, moist, dome-shaped colonies are formed. The organisms are the same in appearance as on serum, but the polar bodies are better marked. In agar stab cultures, a flat, thick, moist, white surface growth with indented edges is formed. The indentations are very evident after 48 hours' growth. Along the needle track the growth is well marked. No growth was obtained on *gelatin*. On *potato* in 24 hours a very extensive pale yellow moist growth occurs. The organisms are mostly short, but a number of markedly clubbed and segmented (up to six or eight segments) forms are found. All show large and distinct polar bodies. *Broth* after 48 hours is clear with white rather stringy deposit. In *glucose broth* the deposit is copious and occurs in the form of large white flocculent masses which tend to stick to the sides. The reaction is very acid. Non-pathogenic.

This organism differs slightly from the diphtheria bacillus in morphology though somewhat resembling the uniformly staining type (Plate VI, fig. 6), and also differs in its growth on broth and potato. It is from its marked growth on the latter that the name has been given. It resembles the organism next described very closely, except in its growth on gelatin, potato, broth and glucose broth. It may be the same organism as that described by Abbott (1891).

6. *Bacillus diphtheroides brevis.* (Plate XV, fig. 4.) Graham-Smith (1904).

Origin. Obtained from a large abscess cavity opening into the mouth.

The colonies on *serum* in 24 hours are small, smooth, and white, but not so well raised as those of the diphtheria bacillus. Subsequently they develop somewhat filmy edges. The organisms in shape resemble Hofmann's bacilli, but are slightly curved and clubbed and show segmentation. The segments stain darkly, but the intervening bands except the middle one are not very definitely marked. They are non-motile, retain Gram's stain, and show polar bodies by Neisser's method. In subsequent subcultures segmentation is a marked feature. On *agar* stroke cultures an abundant, white, soft, slimy growth is produced in 24 hours. The organisms are long, well curved, clubbed and segmented, and show good polar bodies resembling the diphtheria bacillus shown in Plate VI, fig. 4. Agar stab cultures show abundant white slimy surface growth which frequently becomes after further growth coarsely granular. A confluent, abundant growth occurs along the needle track with numerous projecting colonies. On *gelatin* in three days, medium-sized, round, smooth, dry-looking colonies with raised centres develop. On gelatin stab cultures a large, white, dry, granular surface growth is formed, and small round colonies develop in the needle track. On *potato* in 24 hours an extensive growth, thick, soft, and cream-coloured, is formed which gradually becomes granular and slightly yellowish. The organisms vary in appearance, some are short, but many long and well segmented with good polar bodies. In 48 hours *broth* becomes slightly cloudy and there is a fine whitish granular deposit. The reaction of *glucose broth* is extremely acid. *Milk* becomes partially coagulated in a few days and *indol* is produced. It is non-pathogenic.

This organism differs from the diphtheria bacillus in its growth on agar and potato, and somewhat in its morphology.

7. *Origin.* Obtained from the throat of a healthy child. (Graham-Smith, unpublished.)

After 18 hours' growth the colonies or *serum* are large, smooth, moist and yellow. The organisms are of medium length, slightly curved and have a central light band. In some the ends are oval or club-shaped, in others pointed. All are characterised by having an enormous polar body at each end. On *agar* minute, round, flat, delicate slightly granular, gray colonies are formed and the bacilli are segmented and exactly resemble diphtheria bacilli. *Broth* cultures show a thick adherent film and a yellow stringy deposit, and the fluid is slightly clouded. *Glucose broth* becomes acid. A good growth of small moist white colonies occurs on *gelatin*. After 24 hours there is a slightly raised yellowish growth on *potato* which in a few days becomes very abundant, dry and yellow. Non-pathogenic.

8. *Origin.* Obtained from a case of diphtheria.

On *serum* an abundant creamy growth occurs which later becomes yellow. After 18 hours' growth the organisms appear as short wedge-shaped and swollen rods, which do not show granules by Neisser's method. On *glycerine agar* and *potato* an abundant yellow growth occurs. Broth is made acid. Non-pathogenic. (Hamilton, 1904, No. 10.)

9. *Origin.* From the throat of a patient suffering from diphtheria.

On *serum* produces a moist colourless growth, on which medium the organisms appear as slender, barred and uniformly stained rods. They show no granules by Neisser's stain. On *glycerine agar* there is abundant growth and the agar turns brown. On *potato* an abundant gray shining growth is produced. *Broth* becomes cloudy and finally acid. Non-pathogenic. (Hamilton, 1904, No. 15.)

Eyre and Flashman (1904, p. 1109) isolated from the throat of an insane patient, a bacillus corresponding in all respects to an organism previously found by the former (1900) in milk, named "Diphtheroid No. 1." (See p. 329.)

E. *Organisms differing from the diphtheria bacillus in all their main characters except morphology.*

10. *Origin.* Obtained from the throat of a healthy child. (Graham-Smith, unpublished.)

After 24 hours' incubation the colonies on *serum* are still very small, but after 48 hours they become large and show a well raised, granular, irregular, opaque centre surrounded by a large, thin, granular zone with irregular margin. The bacilli are long and curved and occasionally clubbed and many show several bands. Terminal polar bodies are well marked. Although any single organism is indistinguishable from the diphtheria bacillus, masses are occasionally seen with the bacilli lying in parallel rows unlike diphtheria bacilli. On *agar* round, white, smooth, moist raised colonies are produced. There is no growth on *gelatin*. *Broth* cultures show a marked surface film of scale-like detached pieces, and a deposit of large flocculent granules. The fluid remains clear. In *glucose broth* no acid is formed. On *potato* there is a slight yellowish growth, and many of the organisms are markedly curved and show numerous segments. Non-pathogenic.

11. *Origin.* From the throat of a scarlet-fever patient.

On *serum* an abundant colourless growth is produced. After 18 hours' growth the organisms appear as slender, solid and long granular rods, and after further

DIPHTHERIA-LIKE BACILLI FROM SERUM CULTURES.

Fig. 1.
Hofmann's bacillus (Pseudo-diphtheria type). First culture 24 hours.

Fig. 2.
Typical Hofmann's bacillus. Subcultures from Pseudo-diphtheria type 24 hours.

Fig. 3.
Bacillus diphtheroides citreus. First culture 24 hours.

Fig. 4.
Bacillus diphtheroides brevis. First culture 24 hours.

Fig. 5.
Bacillus maculatus. First culture 24 hours.

Fig. 6.
Bacillus diphtheroides liquefaciens. First culture 24 hours.

[From Graham-Smith, *Journ. of Hygiene*, Vol. IV. 1904.]

growth are like typical diphtheria bacilli. On *glycerine agar* and *potato* abundant colourless growth is produced. *Broth* becomes cloudy and remains neutral. Non-pathogenic. (Hamilton, 1904, No. 4.)

F. *Organisms differing from the diphtheria bacillus in most of their main characters except morphology, and capable of liquefying serum and gelatin.*

12. *Bacillus diphtheroides liquefaciens.* (Plate XV, fig. 6.) (Graham-Smith, 1904.)

Origin. Found in considerable numbers in the mouth of a patient suspected to be suffering from diphtheria.

On *serum* minute rounded colonies are formed in 24 hours, but in 48 hours they are medium-sized, round, slightly yellowish, dome-shaped and opaque. After 10 days' growth the colonies have sunk into slight pits, and the medium become partially liquefied after being kept 20—30 days at room temperature. The organisms are very long, and markedly curved, and lie in groups more or less parallel to one another. There is very little clubbing, and but slight signs of segmentation, but all show well marked terminal and other polar bodies. Some specimens remain as unstained shadows. They are motile, but the movements are slow, and they retain Gram's stain. These organisms bear a fairly close resemblance to the diphtheria bacilli shown in Plate VI, fig. 5. On *agar* stroke cultures in 24 hours a thick, moist, smooth, slightly yellow, abundant growth is formed. The appearance of the organisms is the same as on serum. On the surface of agar stab cultures an extensive moist smooth growth occurs, which occasionally in old cultures shows concentric markings. In the depth the colonies run together to form a continuous growth. Some of the discrete colonies at the edges are rounded, but have blunt projections. On *gelatin* very minute, almost transparent colonies are formed. The medium becomes liquefied round them in about 10 days. In a few days liquefaction is complete with a whitish-yellow mass lying in clear fluid. On gelatin stabs in 3 days the small yellowish surface growth is lying in a small cup-shaped area of liquefaction. In 11 days there is a deep funnel-shaped hollow with yellowish growth at the bottom and very minute colonies along the lower part of the needle track. On *potato* in 3 days a thin extensive white growth is formed, which in 6 days is very abundant and yellow. The organisms are of medium length, markedly curved, thin and stain uniformly. Many are clubbed and the polar bodies are very minute. *Broth* in 48 hours is slightly cloudy, with a large deposit of finely granular matter. In 48 hours the reaction of *glucose broth* is neutral or faintly alkaline. In 6—8 days *litmus milk* is decolorised and firmly clotted. No *gas* is produced, but much *indol* is formed and nitrates reduced. It is non-pathogenic.

G. *Chromogenic organisms.*

13. *Origin.* From the throats of two cases of scarlet fever.

On *serum* there is an abundant growth which liquefies the medium. The bacilli are short solid rods with a few small granules, but show no granules by Neisser's method. On *agar* an abundant green growth is formed and the agar turns purple. On *potato* a brownish growth is formed. *Broth* becomes cloudy and acid. Non-pathogenic. (Hamilton, 1904, Nos. 16 and 17.)

H. *Diphtheria-like bacilli virulent to animals.*

14. Davis (1899) isolated from the mouth in certain cases of scarlet fever a short diphtheria-like non-motile bacillus, which in doses of 2 c.c. injected subcutaneously produced a general septicaemia and peritonitis in guinea-pigs without oedema or congestion at the site of inoculation. Guinea-pigs which received antitoxin died more quickly than those which did not. The organism does not form a toxin. "Morphologically, it bears a striking resemblance to the diphtheria bacillus. It is polymorphic, sometimes short, rather evenly stained, while the individuals lie in parallel lines; at other times it is long, lies irregularly, and gives the broken stain typical of *B. diphtheriae*." It stains by Neisser's and Gram's methods. On *serum* and *broth* the growth is like that of the diphtheria bacillus. On *agar* it produced colonies with dark centres and uneven edges, and an acid reaction in *glucose broth*. A. Williams (1898) also seems to have isolated the same species from a patient who had suffered from diphtheria six weeks before.

15. Ruediger (1903) has recently described an organism which he calls a virulent pseudo-diphtheria bacillus.

Seven strains of this organism were isolated from seven fatal cases of scarlet fever with gangrenous tonsilitis. Diphtheria antitoxin had no influence on the patients, and in fact in two cases its injection appeared to hasten death. The bacillus is described as resembling the true diphtheria bacillus in morphology, causing "uniform turbidity in *broth*, a soft, moist, and whitish growth on *agar*, a hardy light brown growth on *potato*, and turning litmus *milk* white in 5—6 days." Apparently all strains showed polar bodies by Neisser's method, and were agglutinated in dilutions of 1 in 200 by the serum of a rabbit which had received three injections of 24 hour broth cultures of strain No. 5. "Guinea-pigs are not protected against this organism by anti-diphtheria serum. All seven strains are pathogenic for guinea-pigs after having been kept on agar for several months, when injected intraperitoneally in doses of 4 to 5 c.c." At the autopsies "the serous cavities contained a moderate quantity of fluid. The liver, spleen, and kidneys were markedly congested. The organisms could be isolated from the peritoneal cavity, heart's blood, and internal organs." A protective serum for guinea-pigs against injections of living cultures was obtained from a rabbit.

Hamilton (1904) continued Ruediger's investigations and obtained several other examples of this organism[1]. In testing for virulence she used intraperitoneal

[1] On some occasions the cultures of these organisms appear only to have been obtained with difficulty. Hamilton describes her method of securing cultures in the following words: "It happened several times that serum cultures 24—48 hours old, from a throat the smears from which had shown the presence of suspicious bacilli, would fail to show anything but cocci, and the cultures would then be rejected. Happening to re-examine such a culture after seven days, I found it full of slender straight and curved rods with bipolar granules. After that the cultures were made on glycerin agar as well as on serum, and, in case bacilli were found on neither, a loopful of the serum culture was transferred to a tube of broth, and from this tube a loopful to the water of condensation at the bottom of a tube of glycerin agar, and the tube tipped to allow the water to run over the inclined surface of the agar. From such a culture colonies of bacilli could usually be obtained, and, in case of failure, the original serum culture was allowed to stand at room temperature for four or five days, and the procedure repeated. By this means it was found possible to isolate bacilli in all cases in which the original smears had shown their presence."

injections of broth cultures in doses equal to 1 % of the body weight of the guinea-pigs. In further experiments the dose was diminished according to results. The animals died within 18 to 24 hours, and the following changes were found at the autopsy. "The peritoneum contained a large quantity of clear, reddish fluid, the liver and kidneys were much congested, the latter often dripping blood on section. The spleen was unchanged, and there was no change in the adrenals. The bacilli could be isolated from the peritoneal and pleural fluids, the blood, the urine, and all the organs, and from the subcutaneous tissue in a few cases. When a bacillus has proved virulent to guinea-pigs two further experiments were made with it. A dose of diphtheria antitoxin, sufficient to protect against a lethal dose of diphtheria bacilli, was given to one guinea-pig together with the ascertained lethal dose of the organism in question. At the same time a second guinea-pig was given a lethal dose of the organism, and also a sufficient quantity of the serum of a rabbit immunised against Ruediger's bacillus to protect against a lethal dose of the latter." "The experiments show that diphtheria antitoxin has no power to protect against these organisms and in some cases even seemed to hasten death and Ruediger's serum has no power to protect against diphtheria bacilli."

Apparently at least two distinct species of organisms whose pathogenic properties are neutralised by Ruediger's serum were isolated, one from an ear discharge (see No. 29).

Of the first species seven examples were obtained from various lesions of the mouth some of which were diagnosed clinically as diphtheria. After 18 hours' growth on serum five are described as short, and two as like typical diphtheria bacilli. Four showed polar granules by Neisser's method. Most of them gave abundant growths on all media, but in the case of one the growths were scanty. All except one produced acid in broth, and in most the medium remained clear. In all cases animals were protected by Ruediger's serum, but not by diphtheria antitoxin.

Hamilton and Horton (1906) made further investigations on this group of organisms. They found that all their strains of typical diphtheria bacilli haemolysed rabbits' corpuscles, while all their typical pseudo-diphtheria bacilli did not. Only two out of 18 strains of virulent diphtheria-like bacilli, whose effects were not neutralised by antitoxin, haemolysed the corpuscles. Their most interesting observations are on the specific bacteriolysins produced in the blood of rabbits immunised against these bacilli. One of these strains was injected subcutaneously into rabbits at 7 day intervals beginning with a dose of 1 c.c., and finally reaching 12 c.c. The method of testing the bactericidal action was as follows—five tubes were used:

No. 1 contained 1 c.c. of plain broth.
No. 2 „ ·8 c.c. of broth and ·2 c.c. of normal serum.
No. 3 „ ·9 c.c. „ ·1 c.c. „
No. 4 „ ·8 c.c. „ ·2 c.c. of immune serum.
No. 5 „ ·9 c.c. „ ·1 c.c. „

After inoculation with the same quantity of culture these tubes were incubated and plates poured after 4, 6, 20 and 24 hours.

The following table, illustrating one of their experiments, shows that the serum of the immunised animals exerted a powerful bactericidal action on these races of bacilli, but had no effect on diphtheria or Hofmann's bacilli.

Table showing the effect of normal rabbit serum and the serum of rabbits immunised with virulent pseudo-diphtheria bacilli upon these organisms, diphtheria bacilli and Hofmann's bacilli.

	Normal rabbit serum				Immune rabbit serum							
	Serum 0·2 Broth 0·8		Serum 0·1 Broth 0·9		Serum 0·2 Broth 0·8		Serum 0·1 Broth 0·9		Serum 0·05 Broth 0·95		Control Broth 1 c.c.	
Bacilli	6 hrs.	24 hrs.	6 hrs.	24 hrs.	6 hrs.	24 hrs.	6 hrs.	24 hrs.	6 hrs.	24 hrs.	6 hrs.	24 hrs.
[1] Bacillus No. 1	6000	∞	6000	∞	0	1	27	35	50	496	∞	∞
,, No. 2	4224	∞	5000	∞	24	1	34	6	26	0	4000	∞
,, No. 3	400	7000	200	7100	25	700	8	600	30	330	560	6000
,, No. 4	2000	∞	2510	∞	0	0	200	0	–	–	8000	∞
Diphtheria bacillus 1	23	∞	21	∞	10	∞	37	∞	–	–	17	∞
,, ,, 2	570	3223	110	1616	800	3440	750	3880	850	4160	100	440
Diphtheria-like bacillus	1760	∞	–	–	1840	∞	–	–	–	–	1600	∞
[2] Hofmann's bacilli ? 1	560	–	600	840	720	440	2000	1000	–	–	4500	10000
2	600	∞	400	∞	350	∞	350	∞	480	∞	8000	∞
3	800	∞	8000	∞	8000	∞	8000	∞	8000	∞	750	∞
4	1440	∞	880	∞	1280	∞	720	∞	–	–	2000	∞

They observed that the bactericidal substance was very stable resisting the action of age, light, drying and heat (80—90° C.) to an unusual degree. The active principle is removed by filtration through porcelain. They also note that the immune serum contains an agglutinin, and that phagocytosis plays an important rôle in the protection of guinea-pigs against these bacilli.

As the result of their experiments they conclude that "the bacilli described form a distinct group from *B. diphtheriae* on the one hand, and other pseudo-diphtheria bacilli on the other. The distinction lies in the fact that goats and rabbits, immunised against one member of this group, yield serum which is bactericidal to other members of the group, but not to *B. diphtheriae* or other pseudo-diphtheria bacilli, and which contains opsonin specific for members of this group."

16. Hamilton (1904) also obtained another species of virulent diphtheria-like bacillus from one case diagnosed as diphtheria and two cases of measles. This organism killed guinea-pigs in the same manner as those just described, but the animals could be protected either by diphtheria antitoxin or Ruediger's serum.

After 18 hours' growth most of the organisms are short, but long and barred forms occur in some cultures. No granules are shown by Neisser's method. They

[1] Bacilli Nos. 1 and 2 were isolated by Ruediger from the throats of scarlet-fever patients, No. 3 by Hamilton from the throat of a patient suffering from laryngitis, and No. 4 from the urine of a scarlet-fever patient.

[2] These organisms are described as "ordinary pseudo-diphtheria bacilli," but are not otherwise defined. They were derived from cases of otitis media, conjunctivitis, and two cases of scarlatinal sore throat.

produce abundant white or yellowish growths on all media, and *gas* in glucose agar. *Broth* is made cloudy and in some cases becomes acid.

I. *Streptothrices resembling diphtheria bacilli.*

17. Gordon (1903, No. 10) (Plate XIV, fig. 3) describes a streptothrix whose broken fragments closely resemble diphtheria bacilli, being slightly curved, showing polar granules, and retaining Gram's stain. This organism does not grow on *serum* but produces a good growth of raised, granular, well defined colonies on *agar*, which adhere to the medium. On *gelatin* a gray, slightly raised growth is produced which becomes wrinkled transversely, and begins to liquefy the medium on the 14th day. A faint turbidity and a slight sediment are produced in *broth*, and *glucose broth* is made acid. *Milk* is not altered. Non-pathogenic.

18. This organism was found very abundantly in the throat of a child suffering from a slight sore throat, and was at first mistaken for a diphtheria bacillus by an experienced bacteriologist. (Graham-Smith, unpublished.) The majority of forms met with in microscopic preparations are almost indistinguishable from diphtheria bacilli, being curved, slightly clubbed, and segmented and show well marked polar bodies. Small masses are, however, occasionally seen in which long thread-like forms are encountered, many times the length of the bacillary forms. These threads are arranged in interlaced masses, and each thread shows numerous polar bodies at intervals corresponding to the length of the bacillary forms. Branching specimens are occasionally seen. On *agar* the majority of specimens are exactly like diphtheria bacilli, but long thread-like forms are commoner than on serum. On *potato* most of the organisms are short, stain poorly, and are much swollen at one or both ends. Long filaments with swollen ends are occasionally encountered. Growth on *Loeffler's medium* or Cobbett's modification of Lorrain-Smith's medium is poor, but the organism grows well on solidified *calf's serum*. The colonies are just visible after 24 hours' incubation, and after three days' growth have attained the size of small diphtheria colonies. The smaller colonies are round but the large ones are somewhat irregular in outline, and the surface in all is slightly wrinkled and granular. The colonies adhere to the surface and can seldom be removed without taking up some of the medium. On *agar* after three days' incubation the colonies are white, granular, dry looking and raised above the surface. Their edges are extremely irregular and deeply indented, and the growth adheres firmly to the surface. No growth occurs on *gelatin*. *Broth* remains clear without any surface film, and a coarse granular deposit forms on the sides and bottom. A small amount of acid is formed in *glucose broth*. On *potato* the growth is invisible. The organism is non-pathogenic to guinea-pigs when injected in large doses either subcutaneously or intraperitoneally.

The bacillary forms of *Streptococcus scarlatinae* may so closely resemble diphtheria bacilli as to cause difficulty in diagnosis. (Gordon, 1900–1, Case VII. p. 365.)

Diphtheria of the nose.

That diphtheria may affect the nose has been well known for a long time. The disease may be confined to the nose, or the throat and nose

may be attacked at the same time. It is also well recognised that nasal diphtheria may vary greatly in the severity of its manifestations, not infrequently being of so mild a nature as to be mistaken for a mild catarrh. Several instances of cases of this nature giving rise to severe faucial diphtheria have already been quoted (p. 310), and further statistics need scarcely be given. There are, however, some manifestations of nasal diphtheria which require special mention, namely (*a*) chronic fibrinous rhinitis, and some forms of (*b*) atrophic and (*c*) external rhinitis.

(*a*) *Fibrinous rhinitis.*

The name chronic membranous or fibrinous rhinitis has been applied to a certain class of cases of diphtheria in which the disease principally or solely affects the nasal mucous membrane, and is attended by little or no constitutional disturbance. Some writers have claimed that virulent diphtheria is seldom contracted from contact with a case of fibrinous rhinitis, but that on the other hand a case of fibrinous rhinitis has not infrequently been observed to give rise to another of the same kind.

Lieven (1891) indeed reported a case from which he obtained an organism that when introduced into the noses of other children by means of tampons caused a similar condition in them. Some observations on this point have already been quoted (p. 311). As it has been suggested that the diphtheria bacillus concerned in these cases may have a lower degree of virulence than usual, the observations of those workers who have investigated this question have been brought together in the following table. This table, however, in which all those bacilli which killed the inoculated guinea-pigs within five days have been considered as virulent, shows that decrease in virulence is not of common occurrence in this form of the disease, and consequently all cases of fibrinous rhinitis must be considered to be capable of communicating diphtheritic infection.

Lack (1899, p. 13) made the following experiment with a bacillus derived from a case of fibrinous rhinitis. "A large rabbit was tracheotomised and the tracheal mucous membrane rubbed with a pure culture of the bacillus. The animal died on the third day. The whole neck and a portion of the thorax was extremely oedematous with small scattered haemorrhages, and there was a membranous exudation lining the whole trachea and extending down to the bronchi." He further observed that powerful toxins were produced.

Observer	No. of cases investigated	Virulent diphtheria bacilli	Attenuated diphtheria bacilli
Pluder (1896)	6	3	3 ?
Lack (1898)	23	23	0
Neumann (1902)	7	6	1
Ravenel (1895)	7	6	1
Gerber and Podack (1895)	5	5	0
Stamm (1891)	4	4	0
Pugh (1902)	4	4	0
Townsend (quoted by Welch, 1894)	4	4	0
Wolff (1899)	4	4	0
Park (1892)	4	2	2
Morf (1899)	3	3	0
Abbott (1893)	3	2	1
Baginsky (1892)	2	2	0
Concetti (1892)	2	2	0
Dowson (1895)	2	2	0
Cobbett (1901)	1	1	0
Reichenbach (1900)	1	1	0
Abel (1894)	1	1	0
Möller (1902)	1	1	0
	84	76 (90·4 %)	8 (9·5 %)

In consequence of the common occurrence of diphtheria bacilli in fibrinous rhinitis all such cases must be considered to be diphtheritic until the contrary has been proved. Nevertheless all membranous conditions of the nose are not due to the presence of diphtheria bacilli, since in a certain proportion this organism cannot be demonstrated. Gerber (1905) in an investigation of 40 cases only found diphtheria bacilli in 29 (72·5 %), and Meyer (1896) found them in 13 (59 %) out of 22 cases. The latter observer could find no clinical difference between cases due to diphtheria bacilli and cases due to other organisms. Ravenel (1895) in his extensive investigations had previously arrived at the same conclusion. Some of the latter cases are apparently caused by staphylococci or streptococci, and a few by pneumococci (Möller, 1902, Abel, 1892).

(*b*) *Atrophic rhinitis.*

Symes (1903) has recently observed diphtheria bacilli in 20 (87 %) out of 23 cases of atrophic rhinitis; 17 being described as long, and three as short diphtheria bacilli. Morphology in culture was principally relied on. The ages of his patients varied between 9 and 57, and the mean duration of the symptoms was seven years. In one case the condition had been present for 45 years. He examined also a

series of noses of healthy children and adults, but found in them no long diphtheria bacilli. A second control series of noses consisting of cases of ozaena, congenital and acquired syphilis, rhinitis sicca, and lesions other than atrophic rhinitis showed no diphtheria bacilli. Two out of the 17 long diphtheria bacilli found were tested for virulence, and both were found to be virulent. The author does not mention the reaction of the organisms in glucose media, and appears not to distinguish sharply between the diphtheria and Hofmann's bacillus, saying that among the normal noses in 58 % "a short diphtheria-like or pseudo-diphtheria type of bacillus was present in the nose."

(c) *External rhinitis.*

Todd (1898) observed 51 (14 %) cases of rhinitis subsequent to scarlet fever amongst 365 patients at the London Fever Hospital. The early symptoms are redness at the margin of one or both nostrils. Later the redness increases and the surface becomes raw and granular, and crusts are formed on the surface, which may remain from one to five weeks. Pustules were frequently formed on other portions of the body from contact with the discharge. In all 51 cases diphtheria bacilli were found together with staphylococci. None of these cases developed faucial diphtheria although three cases showed diphtheria bacilli in the throat. Pure cultures of the bacilli produced acid from glucose and behaved in all ways like diphtheria bacilli. Out of seven strains injected into guinea-pigs in doses of 1 c.c. six caused death within 60 hours, and one on the ninth day. Two strains injected in doses of ·2 and ·1 c.c. respectively did not kill the animals. Two control animals inoculated with virulent cultures together with antitoxin were not affected. Diphtheria bacilli were also found in the secondary pustules. The author differentiates between the diphtheria and Hofmann's bacillus.

Williams (1901) also calls attention to the occurrence of diphtheria and diphtheria-like bacilli in both clear and thick nasal discharges associated with scarlet fever. Out of 141 cases of post-scarlatinal rhinorrhoea examined, 57 showed bacilli morphologically resembling diphtheria bacilli. Of the 17 strains which he isolated and tested 11 formed acid in sugar broth, but eight of these 11 were totally non-pathogenic while the other three killed the inoculated animals within 24 hours. Control animals treated with antitoxin lived. Six produced no growth in sugar broth. Of these one was pathogenic and five non-

pathogenic. Symes (1895) in a case of similar nature of seven weeks duration also found virulent diphtheria bacilli. Marsden (1901) observed diphtheria bacilli in the nasal discharges of 10 children with slight post-scarlatinal rhinitis. No virulence tests are reported.

The formation of membrane after operations on the nose and tonsils or after the application of the cautery appears to be not very uncommon. Observers, who have investigated these conditions, have seldom noticed the presence of diphtheria bacilli.

Diphtheria-like bacilli from the nose.

Cautley (1896) has described an organism which he has named the *Bacillus coryzae segmentosus*, obtained from the nasal secretion of seven out of eight cases of "influenza cold." Gordon (1903) and Benham (1906) have also isolated this organism and supplemented Cautley's account of it. According to the latter observer this organism is very frequently present in nasal catarrh. He found it in 25 out of 27 cases investigated. Prosser White (unpublished observation quoted by Benham) found it in 17 out of 21 cases. The writer has also observed this organism in nasal secretions and the following account is partly based on these observations:

19. *Bacillus coryzae segmentosus*, Cautley. (Plate XIV, fig. 1.)

On *serum* grows more slowly than the diphtheria bacillus, though the colonies are very similar in appearance. Organisms in 24 hours resemble Hofmann's bacilli with light median bands, but are longer. A considerable number of long forms resembling uniformly stained diphtheria bacilli (Plate XIV, fig. 1) are also found. They are non-motile, retain Gram's stain, and show small terminal polar bodies in most specimens. In subcultures many long segmented forms are seen, with two to four well stained segments, as well as shorter Hofmann-like forms. On *agar* in 48 hours round, smooth, white, raised colonies are formed. The organisms are long, well segmented, curved and clubbed, and but few short forms occur. Polar bodies are only found in a few. They resemble closely the diphtheria bacillus shown in Plate VI, fig. 3. In agar stab cultures in 24 hours a small, white, round, smooth surface growth is formed, which is very extensive after 48 hours. There is well marked growth in the depth. On *gelatin* at 22° C. small, round, white colonies are produced. On *potato* an almost invisible thin whitish growth is formed. *Broth* remains clear, and a *stringy* white deposit is found at the bottom of the tube. *Glucose* broth in 48 hours is very faintly acid. Benham found that it also produced acid from lactose, maltose and saccharose. According to the writer's observations (1906) acid is produced from glucose, galactose and laevulose, but not from maltose, dextrin, glycerine, lactose, mannite or saccharose after 10 days' growth in the serum medium of Hiss. *Milk* in some cases is made acid and clotted. Non-pathogenic.

20. De Simoni (1898) isolated from the nasal secretion of a case of ozaena, a diphtheria-like bacillus 2—4 μ in length. The organism is generally straight, but is sometimes slightly curved. The ends are rounded and one is generally thicker than the other, and the organism is distinctly segmented. Colonies on *agar* resemble those of the diphtheria bacillus. *Broth* becomes clouded and a slight surface growth and granular deposit are formed, and the medium becomes acid. The organism grows well on *serum*, forming large moist white colonies which coalesce, but no growth occurs on *gelatin*. *Milk* is not coagulated and on *potato* growth occurs as a delicate moist broad layer. In *milk* and on *potato* rounded *spores* are formed in the middle of the bacilli. The organism is non-pathogenic. This author (1899) has also described two other species of bacilli from the nose, which, however, do not resemble the diphtheria bacillus very closely in morphology.

Diphtheria of the eye.

Some observers consider diphtheria of the conjunctiva to be a fairly common disease, especially amongst the children in large towns. Burton (1899) and Stephenson (1900) both consider that about 2 % of all cases of ophthalmia in children treated in the Children's Hospitals are diphtheritic, and Gonin (1899) found diphtheria bacilli in seven out of 365 cases of conjunctivitis which he examined bacteriologically. On the other hand many other writers think that diphtheria of the conjunctiva is very rare. Pollock (1905), for example, states that "true diphtheria of the conjunctiva is a very rare condition in this country." The presence of virulent diphtheria bacilli in cases of conjunctivitis has been proved by several investigators, but their records are not sufficiently numerous to base statistics of the incidence of the disease on them. On the other hand many instances have been recorded of the finding of diphtheria bacilli in cases of conjunctivitis without sufficient proof of the identity of the organisms. Inoculation experiments are especially necessary in establishing the identity of diphtheria-like bacilli derived from the conjunctiva owing to the frequent occurrence in this situation of the Xerosis bacillus, which in morphology closely resembles the diphtheria bacillus.

Jessop (1895) was the first observer in England to record the occurrence of virulent diphtheria bacilli in a case of membranous conjunctivitis. He subsequently (1902) proved the presence of virulent bacilli in three other cases. Morax (1895) has recorded virulent diphtheria bacilli in four cases, Uhthoff (1901) in two, and Gordon (1903), Griffith (1901), Eyre (1897), and Guder (1895) in one each. Pilcher (1897) observed diphtheria bacilli in 10 cases, and was able to produce diphtheria of the injured conjunctiva in rabbits and

DIPHTHERIA-LIKE BACILLI FROM SERUM CULTURES.

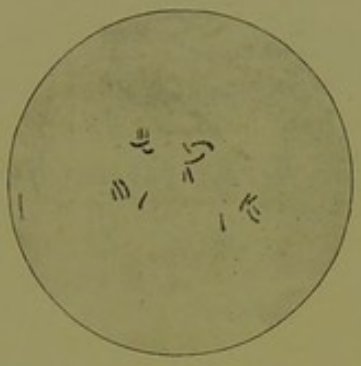


Fig. 1.
Bacillus xerosis.
First culture 30 hours.

Fig. 2.
Bacillus xerosis canis.
First culture 48 hours.

Fig. 3.
Bacillus auris.
First culture 48 hours.

Fig. 4.
Bacillus ceruminis.
First culture 30 hours.

Fig. 5.
Bacillus diphtheroides gallinarum.
First culture 24 hours.

Fig. 6.
Bacillus No. 45, p. 381.

[From Graham-Smith, *Journ. of Hygiene*, Vol. IV. 1904.]

guinea-pigs, with the bacilli derived from some of these. On the other hand Kasatan (1903), Schanz (1897), Moritz (1895) and others have isolated acid-forming but non-virulent diphtheria-like bacilli from similar cases.

Diphtheria-like bacilli from the eye.

21. *The Xerosis bacillus.* (Plate XVI, fig. 1 and Plate IX, figs. 7 and 8.)

The term Xerosis bacillus has been used not only to denote the organism described below which is found both in the healthy and diseased conjunctiva, but has been applied also by some writers to the non-virulent, but otherwise typical diphtheria bacillus. This fact has led to some confusion in the writings on the subject.

As in the case of the Hofmann's bacillus opinions differ as to the relationship existing between the Xerosis bacillus and the diphtheria bacillus. While some hold that the two organisms belong to distinct species, others consider that the Xerosis bacillus is merely an attenuated diphtheria bacillus. Some have asserted that the Xerosis bacillus is capable of giving rise to various lesions of the conjunctiva, and others have denied that it possesses any pathogenic power.

In the following pages the characters and distribution of the Xerosis bacillus are first considered, later evidence for and against its pathogenic powers, and finally its relationship to the diphtheria bacillus.

The Xerosis bacillus on young *serum* cultures closely resembles in morphology the diphtheria bacillus. The bacilli are long, usually curved, and definitely broader at one or both ends than in the middle, and the majority show well marked segments, often very regularly arranged. Preparations made from cultures show the bacilli arranged in the same way as diphtheria bacilli, and Hill has pointed out that they belong to his "snapping" group and undergo the same post-fission movements as diphtheria bacilli. Denny (1903) has studied their morphology after various periods of growth, and remarks that after 8—12 hours' incubation the organisms stain uniformly and resemble Hofmann's bacilli. After 15 hours they are longer and show signs of segmentation, while after 24 hours they are still longer and the segmentation is more marked. After 36 hours segmentation is very well marked and the organisms are yet longer. While some observers state that they show polar granules by Neisser's method others state that they do not. This is probably due to the fact that polar bodies are not commonly found in many individuals in the original cultures, but are more common in subcultures. They are non-motile and retain Gram's stain. All observers agree that they grow more slowly on serum than do diphtheria bacilli. After 24 hours' incubation the colonies are still very minute, and even after 48 hours are smaller than those of young diphtheria bacilli. At this time the colonies are rounded, rather flattened, and have a dry, scaly appearance, and a gray colour. They further differ from the colonies of the diphtheria bacillus in adhering firmly to the surface of the medium. The edges of the colonies are uneven and the irregularity becomes more marked after further growth.

On *agar* minute, raised, round, smooth, almost transparent colonies make their appearance in 48 hours, which adhere closely to the medium. In *agar stabs* the

surface growth is small and almost transparent, and very minute colonies are seen along the line of puncture. On this medium the organisms are broader than on serum, well segmented, but show no polar bodies. On *gelatin* a few strains only show any growth as very minute, round and transparent colonies. On *potato* growth occurs as an invisible film. *Broth* remains clear and growth occurs in the form of large or small granules which are found at the bottom of the tube. There is no surface growth. According to most observers there is no acid production in *glucose broth*. Knapp (1904) studied the action of 10 strains of this organism on 1 % solutions of various sugars in the serum medium of Hiss, and stated that they produced acid with dextrose, mannite, and saccharose, but not with dextrin.

Benham (1906) on the other hand states that it does not produce acid with glucose, lactose, saccharose or maltose.

According to the majority of observers it is entirely non-pathogenic to animals, but a few have stated that subcutaneous injection gives rise to a slight oedema. The Xerosis bacillus therefore differs from the diphtheria bacillus in the rate of growth, and in the appearance of its colonies on serum. It also differs in producing little or no acid in *glucose* broth, and in its entire lack of virulence.

The Xerosis bacillus was first discovered by Kuschbert and Neisser (1884) in Xerosis of the conjunctiva, and received its name from that disease. Subsequent observers have frequently found it in this disease. Stephenson (1898) examined clinically 6209 London school children and found that 1·87 % were suffering from Xerosis, and remarks that he has never failed to find the Xerosis bacillus in this condition. It has also been recorded in conjunctivitis of every description. Pollock (1905) observed "that colonies of the Xerosis bacillus were almost invariably present in culture tubes taken from muco-purulent conjunctivitis," and Griffith (1901) found it in 17 out of 20 cases of follicular conjunctivitis and in six out of 12 cases of slight conjunctivitis. It has also been recorded by various observers in true and chronic trachoma and several other conditions. Deyl (1893) isolated it in 15 cases of chalazion formation and considered it the cause of the disease, and stated that he had produced such formations in the eyes of animals by inoculation of the bacillus.

Although Franke (1893), Eyre and others have failed to find this organism in the normal conjunctiva there is ample evidence from the experiments of recent observers that it is frequently present.

Griffith (1901) found it very frequently, stating that from one loop passed over the conjunctiva 30 to 100 colonies were commonly obtained on serum cultures and once over 200 colonies grew. He concludes by observing that "the organism known as the Xerosis bacillus is the most common inhabitant of the conjunctival sac." Coppez (1899) states that it is an organism commonly found in the secretions of the eye, and

Lawson (1898) considers it to be "the most common and most universal inhabitant of the conjunctival sac." Heimersdorff (1898), Stephenson (1898), Fraenkel (1897) and Uhthoff (1896) also found it very frequently. The relative frequency of the occurrence of the Xerosis bacillus in the normal conjunctiva has been the subject of very few observations. Griffith (1901) in 210 examinations found it 120 (57·1 %) times, Lawson (1899) in 200 examinations 118 (59 %) times, and McKee (1906) in 140 examinations 42 (30 %) times. The writer has observed it in almost all the normal eyes he has examined.

The experimental evidence, as far as it goes, is entirely against the pathogenicity of this organism both towards men and the lower animals.

Piltz (1890), Braunschweig (1890), Fraenkel and Franke (1887), Lachowiez and others have inoculated the organism into the human conjunctiva without setting up any disease.

Stephenson (1898) made three sets of experiments on the human eye: "First, attempts to set up Xerosis in the second eye of a patient with one eye already affected, morsels of greasy material from the conjunctiva being used for the purpose; second, attempts to produce Xerosis in the eye of a second person by conveying to it some of the frothy material obtained from another subject; and third, attempts to set up the condition by inoculating healthy conjunctivae with pure cultures of Xerosis bacilli." All these experiments were without result. Many attempts have been made by numerous investigators to produce lesions in the eyes of animals with pure cultures of this organism, obtained from the normal and also from the inflamed eye, but all have failed. The methods employed have been inoculation onto the surface and under the conjunctiva and into the anterior chamber of the eye in dogs, rabbits, guinea-pigs and mice. Intravenous, intraperitoneal and subcutaneous inoculations even in large doses also produce little, if any, effect on the ordinary laboratory animals. The exceptional observation of Deyl (1893) has already been quoted (p. 364).

Owing to its general resemblance both in morphology and culture to the diphtheria bacillus, the Xerosis bacillus has been regarded by some observers as being a diphtheria bacillus which has lost its virulence, and it has even been suggested that the Xerosis bacillus may after passing down the ducts into the buccal cavity become under unknown conditions a true virulent diphtheria bacillus—Schanz (1894).

No experimental proof, however, of the relationship of the Xerosis to

the diphtheria bacillus has been brought forward, and those[1] who have specially studied the former organism within recent years have almost without exception taken the view that the two organisms are distinct species.

Other diphtheria-like organisms from the eye.

22. Gordon (1904) obtained from a case of conjunctivitis a non-virulent organism identical with the diphtheria bacillus in morphology and staining properties. (Plate XIV, fig. 2.) On *serum* it formed lemon yellow colonies and on *agar* a dry growth and yellow pigmentation. On *gelatin* the growth resembled that of the diphtheria bacillus, but was yellow. *Glucose broth* became acid. In broth no turbidity was caused and growth occurred as conglomerate crumbs.

Griffith (1901) from his observations on the bacteriology of the conjunctiva considered that it would be possible "to separate from the eye a complete series of bacilli beginning with the short regular form not producing acid in glucose and non-virulent and ending with the typical diphtheria bacillus pathogenic to guinea-pigs."

He describes in detail three diphtheria-like organisms, not including the Xerosis bacillus, all of which are non-pathogenic.

23, 24. Two of these three resemble the Xerosis bacillus closely in all respects, the first (No. II.) differing mainly in producing a light brown growth on serum and the second (No. IV.) (probably a non-virulent diphtheria bacillus) in producing yellowish colonies on agar, and acid in broth lactose and glucose broth.

The third diphtheroid organism (No. V.) differs considerably.

25. Morphologically it is a short oval bacillus with a central unstained interval, showing polar bodies, retaining Gram's stain and producing "irregular soft opaque white colonies on serum, and white opaque spherical colonies on *agar*. *Gelatin* stroke cultures show a raised, opaque, porcelain, white, coarsely granular growth with lateral expansions. *Broth* becomes slightly tinted and has a white deposit. *Lactose* broth becomes slightly and *glucose* broth strongly acid. A little *indol* is produced but no *gas*. *Milk* becomes acid but is not coagulated. On *potato* a dry yellowish white growth occurs. Non-pathogenic.

Diphtheria of the ear.

Various lesions of the ear due to the presence of the diphtheria bacillus have been reported, and several of these reports are accompanied by satisfactory bacteriological evidence. Although the occasional presence of the true diphtheria bacillus in the ear discharges of patients suffering from diphtheria and others has been fully established, the

[1] Peters (1897), Eyre (1897), Stephenson (1898), Heimersdorff (1898), Franke (1898), Axenfeld (1898), Lawson (1899), Griffith (1901), Gordon (1903). Schanz (1899) regards it as a non-virulent diphtheria bacillus.

frequency of its occurrence in these conditions is unknown. Many of the bacteriological reports are unsatisfactory, depending either on a few cultural characters, or on morphology alone, and several of them probably refer to other organisms.

Newsholme (1904) cultivated diphtheria bacilli from the ear discharges of a contact and two convalescents. In one instance the organism was tested and found to be virulent. Graham-Smith (1904) has observed altogether three instances of otorrhoea associated with fully virulent diphtheria bacilli. In one instance the bacilli persisted for 66 days and were virulent when tested on the 53rd day. Gordon (1903) once isolated virulent diphtheria bacilli from an ear discharge. In another case he discovered an organism which differed slightly in cultural characters and was non-virulent. Williams (1901) examined 62 ear discharges of scarlet-fever patients and discovered diphtheria-like bacilli in eight. Of these four were carefully tested, and all formed acid in sugar broth, but produced no effects on inoculated animals. Grixoni (1896) found non-virulent diphtheria-like organisms in a case of violent inflammation of the auditory meatus, and Schilling (1904) observed diphtheria-like bacilli in an ear discharge, which were apparently non-pathogenic to guinea-pigs.

Tobey (1906) obtained from the middle ear a diphtheria bacillus which produced acid with dextrose, but not with lactose or mannite. 2 c.c. of a broth culture injected subcutaneously caused an abscess in a guinea-pig.

Biernacki and Heanley (1906) investigated 24 cases of ear discharge in diphtheria patients. In 20 cases diphtheria bacilli were found. Of the four strains tested for virulence one was virulent and three non-virulent.

Table showing the virulence of diphtheria bacilli found in ear discharges.

Observers	Cases examined	Bacilli virulent	Bacilli non-virulent
Graham-Smith (1904)	3	3	0
Newsholme (1904)	1	1	0
Gordon (1903)	2	1	1
Biernacki and Heanley (1906)	4	1	3
Schilling (1904)	1	0	1
Williams (1901)	4	0	4
Grixoni (1896)	1	0	1
Tobey (1906)	1	0	1
	17	6	11

Diphtheria bacilli have also been reported without any remarks on their pathogenic properties by the following observers:

Scheller (1905) reports diphtheria bacilli in eight cases of post-diphtheritic otitis media. Funke (1901) studied the bacteriology of 76 cases of otitis media and in six he found diphtheria bacilli, in one the xerosis bacillus, and in six pseudo-diphtheria bacilli. He makes no mention of his methods, and his cases seem to have had no connection with diphtheria. He concludes from his observations that diphtheria bacilli are more commonly present in otorrhoea than is generally believed. Green (1901) examined bacteriologically 101 cases of acute suppuration of the tympanum and found diphtheria bacilli in four of them, twice in pure culture. This observer also investigated 144 cases of mastoiditis on which operations were performed, and found diphtheria bacilli in one instance. Councilman, Mallory and Pearce (1900) examined the middle ear in 144 fatal cases of diphtheria and observed diphtheria bacilli in 38 cases. They also cultivated diphtheria bacilli in one instance from an ear discharge. Wolff (1895) examined the middle ear in 22 fatal cases of diphtheria and found diphtheria bacilli in six. Councilman (1894) records the finding of diphtheria bacilli in three cases of otitis media, one following diphtheria, and Marsden (1901) in two cases following scarlet fever. Podack (1895) reported diphtheria bacilli in the ear discharges of two cases following measles, and Jacob (1905) found diphtheria bacilli both in the ear and nasal discharges of a child suffering from diphtheria. Leary (1897) and Kutscher (1895) each record one case.

Diphtheria-like bacilli from the ear.

Graham-Smith (1904) in the discharges from the ears of 10 scarlet-fever patients found on three occasions an organism resembling the diphtheria bacillus, described below as the *Bacillus auris*. From the ear discharges of three of these scarlet-fever cases and from the normal ears of 13 out of 20 persons working in the laboratory, another diphtheroid organism, *Bacillus ceruminis*, was isolated. Of all the ears examined, therefore, 66·6 % contained diphtheroid organisms.

26. *Bacillus auris.* (Plate XVI, fig. 3.)

Origin. From the ear discharges of three scarlet-fever patients.

On *serum* the colonies closely resemble those of the diphtheria bacillus, but grow more slowly. After 48 hours' growth the colonies are medium-sized. After 30 hours' growth the organisms are of various sizes, the majority of over medium length with darkly staining ends. They stain well, showing well marked segments,

with intervening light bands. In the longer forms several segments occur. Nearly all, even the shorter forms, are well curved and have a tendency to be clubbed. A few pear-shaped forms were met with. They are non-motile, retain the stain by Gram's method, and show several well marked polar bodies by Neisser's stain. In subcultures the resemblance to diphtheria bacilli is still more marked. Nearly all the specimens are long, well segmented, and show several good polar bodies. The general arrangement in the field is similar to that of the diphtheria bacillus. On *agar* slopes in 24 hours the colonies are small, round, gray and dome-shaped. The organisms are short, curved and clubbed with well-marked polar bodies. Agar stab cultures in 24 hours show a smooth, moist, white surface growth, and a good growth of discrete round colonies along the line of puncture. On *gelatin* stabs in three days there is an irregular, granular, white surface growth faintly marked by concentric rings, and good growth in the depth. On *potato* in 24 hours there is a slight brownish-yellow growth, which in 48 hours becomes extensive, soft, yellow and glistening. The organisms are short and oval, and stain well with large terminal polar bodies. *Broth* in 48 hours is slightly cloudy with a white stringy deposit. In *glucose broth* a copious, finely granular deposit occurs, and the reaction is very markedly acid. *Milk* remains unchanged. *Indol* is formed. The organism is non-pathogenic.

These organisms closely resemble the diphtheria bacillus in morphology and staining characteristics, but differ, more especially, in their growth on gelatin, potato, and broth.

27. *Bacillus ceruminis.* (Plate XVI, fig. 4.)

Origin. Thirteen specimens were obtained from normal ears, and three from the ears of scarlet-fever patients. Eleven of these were fully investigated. The organisms appeared to be more plentiful when ceruminous secretion was present on the swabs. From this circumstance the name has been given.

On *serum* in 24 hours the growth is scarcely visible, but in 48 hours small, round colonies, indistinguishable from those of the diphtheria bacillus, are formed. After 72 hours the colonies are medium sized to large. Two forms slightly different in morphology were noticed. (*a*) In 30 hours long, thin and curved, and uniformly stained, with small terminal polar bodies. In 72 hours the bacilli are longer, more curved, well segmented, and with well marked polar bodies. (*b*) Medium length, slightly curved, uniformly stained, but markedly clubbed, with large polar bodies in a few specimens. After 72 hours the appearance of the two forms is similar. These organisms are non-motile, and retain the stain by Gram's method.

On *agar* after 24 hours the colonies are small, round, gray, and dome-shaped, but later become large and white. The organisms are of medium length, curved, often clubbed, fairly well segmented, and show good polar bodies. On agar stabs a small, white, moist surface growth is present in 24 hours, which after 48 hours' growth is often lightly marked with concentric rings. A confluent growth takes place in the line of puncture.

No growth was obtained on *gelatin*. On *potato* in 24 hours a whitish to yellowish-white growth occurs, later becoming abundant and yellow. After about 10 days the growth has a dry granular appearance. The organisms are mostly short, slightly

curved, and clubbed. *Broth* remains clear and a small, stringy, white deposit is formed. Cultures of this organism in the serum medium of Hiss containing glucose, galactose, lactose, dextrin, laevulose, maltose, glycerine, mannite and saccharose form no acid even after 10 days' cultivation. In *glucose broth* the deposit is granular and the reaction neutral or alkaline. *Milk* remains unchanged and no *indol* is formed. They are non-pathogenic.

In morphology and staining characters these organisms closely resemble the diphtheria bacilli shown in Plate VI, fig. 6, but differ in the rate of their growth on serum, their characters on agar, potato and broth, and in producing no acid in glucose broth and no growth on gelatin.

"The results of these observations show that a species of non-pathogenic organism, almost indistinguishable from the diphtheria bacillus in morphology, is present in the majority of normal ears, and that another species also resembling the diphtheria bacillus is frequently present in the ear discharges of scarlet-fever patients. Consequently it seems highly probable that many of the organisms found in ear discharges and considered to be diphtheria bacilli, belonged to other species."

28. Hamilton (1904, No. 13) isolated from the ear discharge of a scarlet-fever patient an organism, which showed Neisser's granules, and in morphology closely resembled the typical diphtheria bacillus. It produced an abundant creamy growth on *serum*, and liquefied the medium. On *glycerine agar* there was an abundant colourless growth, and on *potato* an abundant yellow growth. *Broth* remains clear and becomes acid—non-pathogenic.

29. Hamilton (1904) also obtained a diphtheria-like bacillus in pure culture from the ear of a scarlet-fever convalescent who developed otitis media. After 18 hours' growth on *serum* short and long deeply staining forms as well as some barred and a few granular forms occur. The granules are well exhibited by Neisser's method. Serum is rapidly liquefied, and a purple growth is produced both on *agar* and on *potato*. *Broth* becomes acid and is not rendered cloudy. Injections of pure cultures rapidly kill guinea-pigs. They are protected by Ruediger's serum, but not by antitoxin.

30. De Simoni (1899) isolated from the discharge in a case of chronic pustular otitis slightly curved bacilli with rounded ends, which showed well defined polar granules but did not retain Gram's stain. In young cultures a few wedge-shaped forms were seen, but in old cultures they became more numerous. On *serum* small, raised, moist, white colonies, and on *agar* large, white, smooth colonies are produced. Good growth occurs on *potato*. *Milk* becomes coagulated in six days, and *broth* becomes clouded with a flaky deposit. They produce acid from glucose and are non-pathogenic.

31. Davis (1899) found in 10 out of 12 cases of otitis media following scarlet fever polymorphic diphtheria-like bacilli, some specimens being long and irregularly stained, others short and evenly stained. The organisms were non-motile and produced whitish colonies with uneven edges and dark centres on *agar*, and rendered *glucose broth* acid, and on *serum* grew like the diphtheria bacillus. Subcutaneous injections of 2 c.c. of broth culture of 9 strains into guinea-pigs produced no effect. One strain was pathogenic (septicaemia) and the animals were not protected by antitoxin.

32. Harrison (1907) isolated a diphtheria-like bacillus from an old case of chronic suppuration of the middle ear. The organism stained by Gram's method, showed polar bodies, and produced acid in *broth*. It differed from the diphtheria bacillus in producing a thick and creamy growth on *serum*, and a thick opaque yellow growth on *agar*. The agar was gradually coloured brown and eventually dark chocolate.

Marzinowsky (1900) obtained a non-virulent diphtheria-like organism from ear wax which retained Gram's stain, and showed polar bodies by Neisser's method.

Warnecke (1900) in two cases of ear disease, and Schilling (1904) in a case of acute otitis media, found non-virulent bacilli closely resembling diphtheria bacilli in other respects.

Forbes (1903) found diphtheria-like bacilli in 32 out of 40 ear discharges, derived from scarlet-fever patients. He was at first inclined to think that these were genuine diphtheria bacilli, but after further investigation abandoned this opinion (1904). Eleven of these bacilli were carefully examined, and all formed acid in *glucose broth*, and the majority produced visible growths on *potato*. Two were pathogenic to guinea-pigs, but at the autopsies none of the usual signs of death from experimental diphtheria were observed. He concludes by stating that "the diphtheroid bacilli differed from each other in many ways and evidently belonged to several groups. These bacilli cannot at present be classified as true diphtheria bacilli, neither do they belong to the commonly recognised varieties of pseudo-diphtheria bacilli."

Gordon (1901) studied seven cases of scarlatinal otorrhoea and found diphtheria-like bacilli in five. In one of these cases a virulent diphtheria bacillus was also found. All the diphtheria-like bacilli were non-virulent to guinea-pigs and all retained Gram's stain. Two strains caused litmus *milk* to become acid, while the other three made it alkaline.

Hamilton (1907) has recently made extensive investigations on the diphtheria-like bacilli found in suppurative processes, especially otitis media, studying 52 examples of miscellaneous suppuration and 142 of otitis media. The results are given in the following table:—

Disease	No. of cases examined	Diphtheria-like bacilli found in	Percentage infected	
Acute scarlatinal otitis media	43	31	72	
Chronic ,, ,, ,,	9	5	55	
Acute non-scarlatinal otitis media	19	4	21	36 %
Chronic ,, ,, ,,	71	11	15	
Miscellaneous suppurations	52	11	21	

"All the cases mentioned in this list as yielding diphtheria-like bacilli contained these bacilli as the predominating organism. Those in which only a few were found were ruled out as doubtful. In nine of the acute scarlatinal cases this bacillus was found in pure culture, in 16 more there were only a few colonies of other organisms."

She states that the 51 strains isolated from cases of otitis media and six from other lesions fall into two clearly defined groups.

Group 1 included 40 strains, which apparently resembled Hofmann's bacillus in morphology and in their growth on agar and potato. They produced acid from dextrose and saccharose, but not from maltose, lactose, or dextrin. Twelve strains

were inoculated into guinea-pigs and of these nine proved to be non-virulent. The other three produced septicaemia and death in 24–48 hours. The animals were not protected by antitoxin.

Group 2 included 11 strains isolated from cases of otitis media. These organisms were long, segmented and clubbed and retained Gram's stain. All produced acid from dextrose and maltose, most (60 %) from dextrin, and a few (10 %) from lactose. Saccharose was not fermented. Four out of seven strains killed guinea-pigs. In one case the control animal was protected by antitoxin.

Hamilton says that "it is evident that there is no hard and fast line between this group and the true Klebs-Loeffler bacillus and when the organisms are non-virulent it is impossible to separate the two."

The *serum* of a rabbit immunised against one type is bacteriolytic for that type, but not for the other. Neither group was acted on by the *serum* of a rabbit immunised against the "Ruediger bacillus."

The discovery of diphtheria-like bacilli in ear discharges has led several recent writers to the conclusion that true diphtheria bacilli are often the cause of these lesions, and that the disease is frequently spread by persons suffering from them. The observations which have just been quoted show, however, that this conclusion is not at present justified, since they prove that diphtheria-like bacilli of various kinds, many of them very closely resembling true diphtheria bacilli and some pathogenic for animals, are very common in these conditions, but that true virulent diphtheria bacilli are rare.

In investigating these conditions it is necessary therefore to prove the identity of the organisms both by cultural tests and by inoculation experiments, including observations on control animals treated with antitoxin, before making a positive diagnosis of the presence of diphtheria bacilli.

The results of those workers who have investigated a series of cases are given in the following table (p. 373).

From this table it can be seen that diphtheria-like bacilli are present in at least 30 % of ear discharges. Of the 61 strains tested on animals 45 (73 %) proved to be non-virulent, six (10 %) were true virulent diphtheria bacilli, and 11 (18 %), though pathogenic, were not true diphtheria bacilli.

Further observations on this subject are required.

Cutaneous Lesions.

The majority of the recorded cutaneous lesions associated with the presence of diphtheria bacilli have occurred in persons suffering from diphtheria. A few instances, however, have been noted of cutaneous

lesions due to the presence of diphtheria bacilli, whose identity has been fully proved, in persons who did not harbour diphtheria bacilli in their throats or noses. So many lesions of the skin, associated with the presence of diphtheria-like bacilli, have been described that it seems scarcely necessary to insist that every means of identification must be employed before organisms, found in such situations, can be considered to be true diphtheria bacilli. In many of the recorded cases, however, very insufficient proofs of the identity of the bacilli have been given.

Table showing the prevalence of diphtheria-like bacilli in ear discharges.

Observer	Cases examined	Diphtheria-like bacilli	Organisms tested on animals	True virulent diphtheria bacilli	Pathogenic—Antitoxin no effect	Non-pathogenic
Davis (1899)	12	10	10	0	1	9
Williams (1901)	62	8	4	0	0	4
Gordon (1901)	7	5	5	1[1]	0	5
Forbes (1903)	40	32	11	0	2[2]	9
Graham-Smith (1904)	13	6	6	3	0	3
Hamilton (1904)	2	2	2	0	2	0
Biernacki and Heanley (1906)	24	20	4	1	0	3
Hamilton (1907)	142	51	19	1	6	12
Wolff (1895)	22	6				
Councilman, Mallory and Pearce (1900)	144	38				
Funke (1901)	76	13				
Green (1901)	101	4				
	645	195 (30·2%)	61	6	11	45

Cutaneous Lesions of the Face and Neck.

Fully virulent diphtheria bacilli have been found by Steffens (1900) in a case of gangrene of the eyelid, by Hála (1900) in an abscess of the eyelid, by Jéz (1895) in the fluid from the vesicles of *herpes labialis* in a patient suffering from diphtheria, and by Dávelos (1894) in the fluid of impetiginous lesions covering the face and neck of a baby.

In a number of other cases diphtheria bacilli have been described, without any record of their pathogenic properties.

[1] Diphtheria bacilli and diphtheria-like bacilli were present in one culture.

[2] The animals showed none of the characteristic lesions of experimental diphtheria.

Councilman, Mallory, and Pearce (1900) found diphtheria bacilli by culture in four out of 10 cervical abscesses developing in the course of diphtheria, and in one case of a membranous condition of the skin near the ear following an ear discharge. Prescott (1898) records a remarkable case. A child recovering from diphtheria developed a swelling in one of the glands in the neck, and a plaster was applied which produced a small blister. A false membrane later formed on the dead epidermis, and cultures showed diphtheria bacilli though none were present at the time in the nose or throat. Todd (1898) records the presence of diphtheria bacilli in a pustule of the face complicating diphtheria, Hayward (1895) in a lesion of the upper eyelid, Kanthack and White (1895) in membranous phagadaenic ulcers of the neck, and Kanthack and Stephens (1896) in several cutaneous membranous sores of the face and neck developing during the course of diphtheria.

Cutaneous Lesions of the Body.

Schottmüller (1895) isolated non-virulent diphtheria bacilli from the pus of an inguinal abscess and from the throat of a boy suffering from diphtheria, and Waelsh (1899) describes diphtheria-like bacilli, pathogenic for rabbits and guinea-pigs, in a serous and pustular eruption of the skin of the trunk. Pitts (1897) records an instance of diphtheria of the umbilicus in a child 14 days old, and Caddy (1893) an instance of diphtheria of the nipple and areola of a woman suckling an infant, which shortly afterwards developed the disease. In the first case no animal inoculations were made, and in the second no bacteriological examination was undertaken. Wright (1894) found diphtheria bacilli in one case of *fistula in ano*, and Müller (1891) in a pustule on the nates.

Cutaneous Lesions of the Hands.

Williams (1902) isolated virulent diphtheria bacilli from a membrane on the finger and from the throat of the same patient, and Abel (1894) from a lesion on the finger of a girl suffering from diphtheria. Müller (1899) and Seitz (1900) have also isolated virulent diphtheria bacilli from lesions of the fingers.

Heelis and Jacob (1906) found diphtheria bacilli in an ulcer on the dorsum of the hand. The patient later infected the outer canthus, and from this the disease spread to the conjunctiva.

A number of cases are also recorded without mention of animal experiments. Tavel (1902), Councilman, Mallory, and Pearce (1900), and Leary (1897) record one instance each, Park, Todd (1898) two cases, Garratt (1904), and Brunner and Hau (1900) three cases, and Wright (1894) seven cases. Two out of Garratt's three cases were suffering from scarlet fever, but diphtheria bacilli could not be demonstrated in the nose or fauces.

Cutaneous Lesions of the Lower Extremity.

Bernard and Jacob (1903) found virulent diphtheria bacilli in gray ulcerating patches on the thigh of a soldier. None were discovered in cultures from the nose and throat. Heelis and Jacob (1906) discovered virulent diphtheria bacilli in two cases of ulceration of the foot, thought to be due to chilblains. Similar organisms, which were not tested on animals, were found in an ulcer of the heel. All these cases occurred at the same time amongst the inmates of an orphanage. Bolton and Brewer (1905) cultivated diphtheria bacilli from a sore, showing a black central scab surrounded by sloughing portions of dirty white skin, on the groin of a girl. No membrane was ever formed. Animal inoculations are not recorded. Todd (1898) briefly records the finding of diphtheria bacilli in two pustules of the foot and two of the toe, complicating cases of diphtheria.

Without mentioning the part affected the discovery of diphtheria bacilli in cutaneous lesions is recorded by Tavel (1903) (abscess), Schick and Ersettig (1903), Gordon Sharp (1898) (two cases), and by Glucksmann (1897) (five cases of eczema). Leary (1896) found diphtheria bacilli in an abscess which developed about a hair follicle two days after an autopsy on a case of diphtheria. The abscess remained local, and a pure culture of the diphtheria bacillus was obtained from it which killed a guinea-pig in 48 hours.

Wound Diphtheria.

Several of the instances of cutaneous diphtheria, which have been mentioned, were due to the infection of insignificant wounds (Park, Pitts, Abel, etc.). Besides these a number of examples of infection of more extensive wounds have been recorded: Tavel (1902) mentions a case of infection of the operation wound for spina ventosa, Brunner (1893) three cases of wound infection, in one of which virulent, and in

the other two non-virulent, diphtheria bacilli were found, Spronck (1892) three examples of infection of tracheotomy wounds. Treitch and Abel (quoted by Flexner, 1895) and Scheller (1905) also mention cases of wound infection.

It must be pointed out, however, that cases of wound infection are not common, and that many of the membranous conditions following the infection of wounds have been proved by numerous observers to be due to other bacteria.

Diphtheria-like bacilli from the skin.

Diphtheria-like bacilli have been frequently described as occurring in various lesions of the skin.

A. *Vaccinia and variola.*

33. Brown (1903) isolated diphtheria-like bacilli from three cases of vaccinia with oedema, but could not find them in uncomplicated cases. The organisms are curved, of irregular width, stain faintly and have rounded ends, and contain darkly stained rounded dots, at intervals. The arrangement of the bacilli is similar to that of the diphtheria bacillus. They retain Gram's stain and show a few polar bodies by Neisser's method. On *agar* grayish-white colonies are produced which coalesce after further growth. In *glucose broth* there is a white deposit and the medium remains neutral. On *potato* a yellowish growth occurs. No growth occurs on *gelatin*, and *milk* is not clotted. One of the inoculated guinea-pigs suffered from a small patch of oedema followed by an ulcer, but another showed no reaction.

34. Nakanishi (1900) found in the pustules of vaccinia in children and calves an organism resembling Hofmann's bacillus in morphology with a central light band and darkly staining ends, which he calls the *Bacillus variabilis lymphae vaccinalis*. On *serum* the colonies are pale yellow, and circular, and grow to a considerable size. On this medium some of the bacilli are short and evenly stained, some are spindle shaped and some segmented, and some have polar bodies. Long segmented bacilli are frequent and branching forms occur occasionally on *agar* and *potato*. Growth on *gelatin* is poor. *Broth* is at first clouded, but later becomes clear with a granular deposit and slight surface film. Good growth occurs in *milk*, and the medium is not coagulated. The growth on *potato* is poor. The organism is pathogenic to rabbits and guinea-pigs, in large doses intraperitoneally, but not to mice.

35. De Simoni (1899) obtained from smallpox pustules bacilli which lie in irregular masses and show small granules in their protoplasm. They are seldom curved and usually have one thick rounded end and one narrower and more pointed end. They retain Gram's stain. Stroke cultures on *serum* after 24 hours show a uniform, moist, lustrous, raised growth, which later becomes thicker. On *agar* the colonies tend to coalesce and produce a thick moist growth. On *gelatin* well marked growth occurs. *Broth* becomes clouded and has a thick flaky deposit.

On *potato* there is a thick, moist, raised, luxuriant growth. *Milk* is not clotted. In *glucose broth* acid is produced. Non-pathogenic.

Besser (1893) and Sanfelice and Malato (1903) have also described diphtheroid organisms in variola, and the latter authors, Neisser (1888) and Laudmann (quoted by Galli-Valerio, 1904) in vaccinia.

36. Galli-Valerio (1904) isolated from *vaccine lymph* a diphtheroid organism, *Corynebacterium vaccinae*, 2—4 μ in length, which shows polar bodies by Neisser's stain, but does not retain Gram's stain. The majority stain evenly by methylene blue, but some show lightly stained patches, and some segmentation. The organism is non-motile, and is apt to be arranged in clumps. On *serum* in 24 hours small white colonies are formed, which later become yellowish. On *agar* small, white, raised colonies surrounded by a clear zone are formed. Later the centre becomes prominent and the edges irregular, and the whole surface becomes covered with irregular bosses. On *gelatin* at 20°C. a similar growth is formed on the surface, but there is very little growth in the depth. No liquefaction occurs. On *potato* a slight grayish white growth occurs. *Broth* at first becomes slightly cloudy, but later becomes clear. A whitish surface growth and flocculent deposit are formed. In *milk* the organism grows well and the medium does not become coagulated. *Indol* is formed, but neither *glucose* nor *lactose* is fermented. Mice and rabbits are not affected by subcutaneous injections. One of the inoculated guinea-pigs developed a small abscess in which the organism was found in pure culture.

37. Klein (1897) isolated from glycerine emulsions of smallpox crust an organism, which he called *Bacillus xerosis variolae*. The organism is rather shorter than the diphtheria bacillus but resembles it closely in morphology. On *agar* very minute colonies are formed which have a raised centre, irregular margin and granular surface. Very little growth occurs on *gelatin*. It is non-pathogenic to rodents and calves.

38. Klein (1897) also isolated from the same material another diphtheroid organism which he called *Bacillus albus variolae*. The bacilli from early agar cultures are very small, but those from older cultures resemble diphtheria bacilli very closely. Amongst these forms a few are to be seen with a conspicuous and deeply stained sheath. On *agar* the colonies are at first small and translucent, but after two days' growth become pure white, large, smooth, raised, and moist looking. The colonies are coherent and satisfactory microscopic preparations are not easy to obtain. Very little growth occurs on *gelatin*. It is non-pathogenic for rabbits and guinea-pigs, but cutaneous inoculations produce vesicular lesions in calves.

39. Levy and Fickler (1900) obtained from calf lymph two varieties of a diphtheroid organism, which they term *Corynebacterium lymphae vaccinalis*. One variety produces a yellowish, and the other a whitish growth on Loeffler's serum. On Loeffler's *serum* a thick, rough, dry growth is formed, and the organisms in young cultures include small wedge-shaped and longer diphtheria-like forms. On *agar* small, gray, irregular colonies with yellowish centres are produced, and a good growth occurs on *gelatin*. *Broth* becomes cloudy and there is a granular deposit. There is no growth in *milk* or on *potato*. Injections cause small abscesses in guinea-pigs and kill mice in 6—7 days with the formation of abscesses.

All the above investigators lay stress on the statement that the organisms they describe belong to the diphtheria group.

B. *Diphtheria-like bacilli in leprosy.*

40. Babes (1899) and a number of other observers have isolated diphtheria-like bacilli from cases of leprosy. Babes obtained them from 12 cases, and Spronck (1898), Levy (1897 and 1899), Czaplewski (1898), Baranikow (1899), Teich (1899), and Kedrowski (1901) have all obtained cultures resembling those of Babes. The discovery by Dean (1905) of a diphtheroid bacillus in rats suffering from a leprosy-like disease is interesting in connection with these observations. (See p. 291.)

The organisms closely resemble diphtheria bacilli in appearance, the majority being long and slightly curved, especially at their extremities. The longer forms also show transverse banding. They stain well with methylene blue, and exhibit metachromatic granules. Growth can be obtained on *serum*, *glycerine agar*, and *agar*, the colonies resembling those of the diphtheria bacillus. They are non-pathogenic to the ordinary laboratory animals, mice, guinea-pigs, rabbits and fowls.

C. *Other skin lesions.*

Peters (1897) observed non-virulent diphtheria-like bacilli in three cases of impetiginous eczema, Paffenholz (1895) in impetigo, Warnecke (1900) in a progressive phlegmonous condition following an ear discharge, and Neisser (1888) in an ulcer of the leg. These authors regarded the organisms they found as Xerosis bacilli.

De Simoni (1899) has also described diphtheria-like bacilli, apparently belonging to different species, in pustules, seborrhoeic eczema, dry eczema, and ringworm. These all formed acid from *glucose*, grew well on potato, and most of them caused clotting in milk.

Bergey (1898) isolated non-pathogenic acid-forming diphtheria-like bacilli, having the same cultural characters as those he cultivated from urine (see p. 386), from the patches of a scaly skin eruption and from an abrasion of the knee. Cobbett (iv. 1901, p. 244) also mentions that he has occasionally met with diphtheroid bacilli from the skin.

Diphtheria of the Female Genital Organs.

Diphtheritic lesions of the female genital organs involving the labia, or vagina, or both, have frequently been recorded, but in most of these cases the diagnoses have been based on clinical signs alone. Many of these records show that diphtheria of the mouth or nose was present at the same time. Even in those instances in which bacteriological examinations have been made, the proofs are in many cases unsatisfactory owing to the lack of animal experiments. The following table summarises the principal records of cases in which bacteriological investigations have been conducted.

Observer	Cases reported showing diphtheria bacilli	Presence in throat or nose	Virulence
Schwab (1904)	2	Present	1 virulent 1 non-virulent
Williams (1902)	1	Present	Virulent
Müller (1899)	1	Absent	Virulent
Freymouth & Petruschky (1898)	1	—	Attenuated
Longyear (1897)	6	Present in one	—
Salmon (1904)	1	Present	—
Erikson (1905)	1	Present	—
Leick (1900)	1	Absent	—
Coues (1897)	1	Present	—
Hewlett & Nolan (1896)	1	Present	—
Bumm (1895)	1	Present	—
Glucksmann (1897)	3	—	—
Favre (1890)	2	—	—
Ware (1900)	1	—	—
Scheller (1905)	1	—	—
Elsner (1898)	1	—	—
Pearce (1898)	1	—	—
Williams (1898)	1	—	—
Cioffi (1897)	1	—	—
Haultain (1897)	1	—	—
Stahl (1895)	1	—	—
Nisot (1896)	1	—	—

Salmon's (1904) case is of especial interest in that patches of membrane were first noted on the labia minora and near the meatus, without local or constitutional disturbance, in a woman admitted into a ward in which an outbreak of diphtheria had occurred. Seven days later during a routine examination of healthy persons diphtheria bacilli were found in her throat. The following day patches of membrane were found on the tongue, lips, palate and mucous membrane of the cheek. The day after a dense white membrane was present on the rectum, vulva and vagina, which a few hours later extended to the cervix. At the same time an abrasion on the cheek also developed a membrane. The patient died 15 days after the lesions were first noticed. Diphtheria bacilli were cultivated from all these lesions.

Of the cases recorded above, six occurred in adults, sixteen in puerperal women, and eight in girls.

Diphtheria-like bacilli from the female genital organs.

41. Foulerton and Bonney (1903) in two cases of puerperal fever isolated diphtheria-like bacilli from the uterus. These organisms are rather longer and coarser than diphtheria bacilli. Some are segmented, others uniformly stained.

They retain Gram's stain but do not show granules with Neisser's stain. They produce a scanty growth on *agar* at 37° C. and after 24 hours' growth show a variety of forms—long beaded, spindle-shaped, pear-shaped, and coccus-like may be seen. Stroke cultures have a surface like ground glass. The growth on *serum* is like that on agar but more opaque. In *broth* it appears as a short evenly staining rod. No acid is produced in *glucose broth*, *milk* remains unchanged, and on *potato* there is a moist spreading layer. This organism differs from the diphtheria bacillus in its coarser shape, and the fact that it produces no acid in glucose peptone broth, and its lack of virulence for guinea-pigs and rabbits. The authors also observed these organisms in cases of catarrh of the cervix uteri.

42. Bergey (1898) isolated from a vaginal discharge a non-pathogenic, acid-forming bacillus corresponding in all respects to the bacillus he isolated from urine.

43. From another vaginal discharge Bergey isolated another segmented diphtheroid organism which produced a thick, moist, yellow growth on *serum* and *agar*, a visible growth on *potato* and a thick yellowish-white membrane on the surface of *broth*. *Glucose broth* became markedly acid after 72 hours' incubation. *Guinea-pigs* inoculated intraperitoneally with large quantities died, but did not show lesions characteristic of experimental diphtheria.

Hallé (1899) found diphtheria-like organisms, varying slightly in morphology, in different parts of the female genital canal, which reminded him of short diphtheria bacilli. All were totally non-pathogenic for guinea-pigs, rabbits and mice. Neisser (1888) isolated diphtheria-like organisms from the vagina, which differed from true diphtheria bacilli in being motile and growing well on potato. Robertson and McRae (v. 1905) isolated bacilli, morphologically identical with diphtheria bacilli, from the leucorrhoeal discharges of a number of female general paralytics.

Many other observers have also briefly mentioned the occurrence of diphtheria-like bacilli in these situations.

Diphtheria of the Male Genital Organs.

Well authenticated cases of diphtheritic lesions of the male generative organs, in which bacteriological examinations have been made, are rare.

McCollom (1897) describes the case of a boy, aged 4 years, suffering from nasal diphtheria who developed a membrane on the prepuce. Virulent diphtheria bacilli were isolated. The same author records a second case in a boy, aged 1 year, suffering from diphtheria of the fauces, who had previously been circumcised. The glans became red and oedematous but no membrane formed. Diphtheria-like bacilli were isolated, which proved non-virulent for guinea-pigs.

Post (1897) quotes the case of an adult who had nursed two cases of diphtheria. He developed a blister on the inner surface of the prepuce which caused acute phimosis. An incision was made and on this a membrane formed, and the prepuce later sloughed. On admission to

the hospital no diphtheria bacilli were obtained in cultures, but diphtheria-like organisms were found in sections of the prepuce. Baranikow (1901) records a case of urethritis in an adult, in the discharge from which virulent diphtheria bacilli were discovered. Munn (1893) quotes the case of a baby, who was circumcised and developed a membrane on the wound, in which diphtheria bacilli were found. Both the mother and brother later suffered from diphtheria. Brunner (1893) discovered diphtheria bacilli in a case of phlegmon of the scrotum.

Diphtheria-like bacilli found in the male urethra.

44. Pfeiffer (1903) examined the urethra in 15 normal men and in 10 suffering from gonorrhoea. He found diphtheroid organisms by cultural methods 11 times in the former group and six times in the latter.

These organisms show a peculiar uneven staining by Loeffler's methylene blue, do not retain Gram's stain, and are neither alcohol nor acid fast. They grow well on *serum*, and produce punctiform, round, grayish white, smooth or slightly granular colonies on *agar* after 48 hours' growth. Growth also occurs on *gelatin*. *Broth* remains clear, and a granular sediment is produced and sometimes a slight surface film. There is a slight acid formation in *neutral broth*. *Milk* becomes acid in 2—7 days at room temperature, and a slight growth occurs on *potato*. *Animal* experiments were negative. Diphtheria agglutinating serum produced no results with six strains in various dilutions between 1 : 20 and 1 : 500.

45. The writer has also recently isolated diphtheroid organisms from the urethra in two cases simulating gonorrhoea, in which the gonococcus was not found, and from one case of gonorrhoea. In smears made from the discharge their morphology varies between short, uniformly staining and long, segmented bacilli. On *serum* colonies are formed after 24 hours' growth like those of the diphtheria bacillus, and the organisms are mostly long and curved and with many segments. Short forms are not uncommon. The majority are rather broader than diphtheria bacilli, and many have enlargements twice as thick as the rest of the bacillus, usually situated about half way between one end and the centre of the bacillus. Distinctly club-shaped forms are also common. They do not possess polar bodies. On *agar* and *glycerine agar* large, white, smooth, moist colonies are produced. A good growth of small, moist, white, round colonies occurs in 48 hours on *gelatin*, and *broth* becomes clouded, and a thin flaky film and large yellow deposit are produced. *Glucose broth* remains neutral. On *potato* an abundant, dry, granular, yellowish growth occurs in 48 hours. Subcutaneous injections of 3 c.c. of 48 hours' broth cultures produce no effect on guinea-pigs. (Plate XVI, fig. 6.)

Foulerton and Bonney (1903) observed in a case of phagadaena of the penis organisms similar to those described in two cases of puerperal fever (No. 41), but did not detect them in 56 cases of urethritis. Eastes (1903) records a similar organism, which was non-pathogenic and did not stain well by Neisser's method, in the discharge of a case of urethritis.

Robertson and McRae (v. 1905) found organisms, morphologically and culturally resembling diphtheria bacilli, but non-virulent, in material taken from the surface of the urethra in 22 consecutive cases of general paralysis in the male.

TABLE SHOWING THE PRINCIPAL CHARACTERS

Morphology	Gram	Neisser	Growth on serum	Growth on agar	Growth on gelatin	Growth on potato
A. Resembling diphtheria bacilli in all their main cultural characters.						
Segmented	+	+	Diphtheria-like	Gray colonies	—	—
,,	+	+	White colonies	,,	Little growth	Invisible growth
Segmented and non-segmented	+	+	Diphtheria-like	White colonies	,,	,,
Segmented	0		White colonies	White round punctiform colonies	,,	,,
,,		+	Diphtheria-like	Diphtheria-like	Diphtheria-like	,,
At first Hofmann-like, later segmented			,,	,,	,,	,,
Segmented	+	+	White	Dry pearly white colonies	Little growth	No growth
,,			Diphtheria-like	Diphtheria-like	,,	,,
,,	+	+	Diphtheria-like or dry	,,	,,	—
Hofmann-like		+	Yellowish colonies	—	,,	Little growth
B. Organisms differing from diphtheria bacilli mainly in the character of their growth on serum.						
Segmented	+	+?	Slow growth, scaly adherent colonies	Small gray adherent colonies	Little growth	Invisible growth
,,	+	+	Opaque white colonies becoming brownish	Small gray granular colonies	White coarsely granular growth	White dry growth
Uniformly staining	+	+	Coherent growth	White coherent	No growth	—
Beaded	+	0	Coherent sharply outlined colonies	—	,,	—
C. Organisms differing from diphtheria bacilli mainly (1) in the non-production of acid.						
Hofmann-like	+	0	Diphtheria-like	White colonies	Fair growth	Invisible growth
Segmented	+	+	Granular adherent colonies	Dry gray growth	—	—
,,	+	+	White filmy growth	White colonies becoming confluent	Colonies white or buff	No growth
Uniformly stained or segmented	0	+	White growth becoming yellow	White	Fair growth	Slight gray growth
Segmented	+	+	Diphtheria-like adherent	Gray	No growth	Invisible growth
,,	+	+	Diphtheria-like	,,	Little growth	,,
(2) in the non-production of acid and the production of						
Segmented	+	0	Diphtheria-like	Moist white	White	Abundant dry yellow
Segmented or uniformly stained	+	0	Scanty growth	Scanty growth, surface like ground glass	—	Moist spreading layer
Segmented	+	+	Semi-transparent, later yellowish colonies	—	White, later citron coloured	Yellowish powdery

Growth on broth	Acid	Gas	Milk: Acid	Milk: Coagulation	Indol	Virulence	Source	Name	Observer	See
Medium clear granular deposit	+					General septicaemia in guinea-pigs. Not protected by antitoxin	Human mouth	—	Davis (1899)	Page No. 354, **14**
Medium clear deposit of large granules	+		0	0	0	Non-pathogenic	,,	*B. maculatus*	Graham-Smith (1904)	349, **1**
Medium clear stringy deposit	+		+	+		,,	Nasal catarrh	*B. coryzae segmentosus*	Cantley (1896)	361, **19**
Medium clear granular deposit	+		+	0		,,	Normal and diseased male urethra	—	Pfeiffer (1903)	381, **44**
,, ,,	+					Produces abscesses in rats and guinea-pigs	Hepatised lungs of rats	*B. muris*	Klein (1903)	291
Medium clear deposit of large granules	+					Slightly pathogenic for young rats	Leprosy-like disease of rats	—	Dean (1905)	291
Medium clear granular deposit	+					Non-pathogenic	Urine	—	Bergey (1898)	386, **46**
Medium clear flocculent deposit	+		+	0	+	,,	Milk	—	,,	328
—	+				+	,,	Mouths of healthy & diseased pigeons	—	Macfadyean & Hewlett (1900)	297
Medium clear granular deposit	?		0	0		Pathogenic in large doses to rabbits and guinea-pigs	Vaccinia	*B. variabilis lymphae vaccinalis*	Nakanishi (1900)	376, **34**
Medium clear granular deposit	+?		0	0		Non-pathogenic	Normal eye	*B. xerosis*	—	363, **21**
,, ,,	+	0	+	0	+	,,	,,	—	Griffith No. IV (1901)	366, **24**
Medium clear coherent masses at bottom	+		0	0		,,	Human mouth	—	Gordon No. 7 (1903)	350, **2**
,, ,,	+		+	+		,,	,,	—	Gordon No. 8 (1903)	350, **3**
At first cloudy, later clear fine deposit	0	0	0	0	+?	Non-pathogenic	See p. 199	*B. Hofmanni*	—	—
Medium clear granular deposit	0	0	0	0	0	,,	Human eye	—	Griffith No. II (1901)	366, **23**
,, ,,	0	0	0	0	0	,,	Milk	"*Diphtheroid III*"	Eyre	329
Medium clear flocculent deposit	0		0	0	+	,,	Vaccinia	*Corynebacterium vaccinae*	Galli-Valerio (1904)	377, **36**
Medium clear granular deposit	0					,,	Normal eyes of dogs & guinea-pigs	*B. xerosis canis*	Graham-Smith (1904)	292
,, ,,	0				+	,,	Mouth of hen	*B. diphtheroides gallinarum*	,, ,,	297
a copious growth on potato.										
Cloud and large yellow deposit	0					Non-pathogenic	Male urethra	—	Graham-Smith	381, **45**
—	0		0	0		,,	Female genitals	—	Foulerton & Bonney (1903)	379, **41**
Medium clear granular deposit	0		0	0	0	,,	Mouth and milk	"*Diphtheroid I*"	Eyre (1900)	329

Morphology	Gram	Neisser	Growth on serum	Growth on agar	Growth on gelatin	Growth on potato
C (*cont.*). **Organisms differing from diphtheria bacilli mainly (2) in the non-production of acid and the**						
Uniformly stained	+	+	Slow growth, colonies diphtheria-like	Gray	No growth	Abundant yellow growth
Segmented	+	+	Raised centre with thin gray surrounding zone	White moist	,,	Abundant dry yellow growth
Beaded	+	+	—	White colonies coalesce	,,	Yellow growth
D. **Organisms differing from the diphtheria bacillus in producing abundant, generally coloured, growths**						
Uniformly staining	+	+	Diphtheria-like	Large white	No growth	Extensive moist yellow
Segmented	+	+	,,	White slimy	Large dry colonies	Extensive cream coloured
Uniformly staining	+	+	Moist yellow growth	Gray colonies	Moist growth	Dry yellow growth
,, ,,		0	Yellow growth	Yellow growth	—	Abundant yellow
Segmented	+	+	Diphtheria-like	Gray	No growth	,,
Uniformly staining	0	+	Moist white growth	Smooth white colonies becoming confluent	Good growth	,,
Hofmann-like	+	+	Soft cream coloured	White	Porcelain white growth	Dry yellowish-white growth
Granular	+	0	Abundant	Thick moist	Good growth	Abundant moist
Segmented			Moist yellow	Moist yellow	—	White
Segmented and uniformly staining forms		0	Yellowish growth	Yellow	Yellow	Yellow
E. **Organisms differing from diphtheria bacilli in liquefying serum or gelatin.**						
Slightly segmented, motile	+	+	Yellow colonies, medium slowly liquefied	Abundant moist yellow growth	Liquefied	Abundant white growth becoming yellow
Segmented	+	+	Gray colonies	Granular, adherent colonies	Slowly liquefied	—
,,	+	+	Abundant creamy, medium liquefied	Colourless growth	—	Abundant yellow growth
,,	+	+	Medium liquefied	Growth slow	No growth	No growth
F. **Organisms differing from diphtheria bacilli in liquefying gelatin and producing soluble pigments.**						
Uniformly stained		0	Medium liquefied	Grayish growth, medium becomes purple	—	Brown growth
Segmented		+	Medium rapidly liquefied	Purple growth	—	Purple growth
G. **Organisms differing from diphtheria bacilli in producing pigmented growths or soluble pigments.**						
"Sheath" form	+	+	Diphtheria-like or confluent	White	Medium made claret coloured	Pink colonies, medium pink
Segmented		0	Colourless growth	Abundant growth, medium becomes brown	—	Abundant gray
,,	+	+	Lemon yellow colonies	Dry yellow	Yellow	—
H. **Organisms differing from diphtheria bacilli in producing spores.**						
Segmented			White colonies which coalesce	Diphtheria-like	No growth	Moist growth

Growth on broth	Acid	Gas	Milk: Acid	Milk: Coagulation	Indol	Virulence	Source	Name	Observer	See
production of a copious growth on potato.										
Medium clear stringy deposit	0		0	0	0	Non-pathogenic	Normal ear	*B. ceruminis*	Graham-Smith (1904)	Page No. 369, 27
Thick surface film, flocculent deposit	0					,,	Mouth	—	Graham-Smith	352, 7
White deposit	0			0	0	,,	Vaccinia	—	Brown	376, 33
on potato.										
Medium clear stringy deposit	+					Non-pathogenic	Abscess of mouth	*B. diphtheroides citreus*	Graham-Smith (1904)	350, 5
Cloudy, granular deposit	+		+	+	+	,,	Mouth	*B. diphtheroides brevis*	,,	351, 6
Thick surface film, stringy deposit	+					,,	,,	—	Hamilton (No. 19)	
—	+					,,	,,	—	,,	352, 8
Cloudy, stringy deposit	+		0	0	+	,,	Ear	*B. auris*	Graham-Smith (1904)	368, 26
Cloudy, flocculent deposit	+			+		,,	,,	—	De Simoni (1898)	370, 30
Cloudy, granular deposit	+	0	+	0	+	,,	Normal eye	—	Griffith No. V (1901)	366, 25
Cloudy with flaky deposit	+			0		,,	Variola	—	De Simoni (1898)	376, 35
White membrane	+					Pathogenic for guinea-pigs in large doses	Vagina	—	Bergey (1898)	380, 43
Cloudy	+	+				Produces septicaemia; antitoxin does not protect	Mouth	—	Hamilton	357, 16
Cloudy, granular deposit	0	0		+	+	Non-pathogenic	Mouth	*B. diphtheroides liquefaciens*	Graham-Smith (1904)	353, 12
—	+			+	+	,,	,,	—	Gordon	350, 4
Medium clear	+					,,	Ear	—	Hamilton	370, 28
Little growth			+	+		Produces subcutaneous abscesses in guinea-pigs	Milk	*B. diphtheroides*	Klein (1901)	328
Cloudy	+					Non-pathogenic	Mouth	—	Hamilton	353, 13
Clear	+					Produces septicaemia; animals not protected by antitoxin	Ear	—	,,	370, 29
Cloudy	+	0			0	Non-pathogenic	Milk	*Diphtheroid II*	Eyre	329
,,	+					,,	Mouth	—	Hamilton	352, 9
Conglomerate crumbs	+					,,	Eye	—	Gordon	366, 22
Medium clear granular deposit	+					Non-pathogenic	Nose	—	De Simoni (1899)	362, 20

Diphtheria of the Anus and Rectum.

Diphtheritic lesions of the anus and rectum are very rare. Salmon (1904) mentions a case in which the patient was suffering from several other diphtheritic lesions. Wright (1894) found diphtheria bacilli in a case of *fistula in ano*, and Neisser (1893) in an inflammatory condition round the anus. Biggs (1893) also mentions an instance of diphtheria of the rectum. In none of the cases was the virulence of the organisms proved.

Diphtheria Bacilli in the Urine.

In a few instances the presence of diphtheria bacilli in the urine has been reported, but in no instance have the bacilli been satisfactorily identified. Barlow (1898) reports the case of a young woman suffering from diphtheria with membrane on both tonsils. On the third day dark coloured urine was passed, and bacilli resembling diphtheria bacilli in morphology and cultural characters were found. No virulence tests were made.

Bujwid (1897) found diphtheria-like bacilli in a sample of urine from a child suffering from tubercle of the kidney. In morphology and cultural characters the organisms resembled diphtheria bacilli, but the subcutaneous injection of 1 c.c. of a ten days' culture only caused strong local symptoms in a guinea-pig.

Diphtheria-like bacilli in urine.

Bergey (1898) isolated diphtheria-like bacilli from urine on several occasions (10). The urine was obtained both from healthy patients and from persons suffering from cystitis.

46. These organisms are short, slender rods showing a tendency to clubbing at the ends, which are stained more darkly. When stained with dilute methylene blue the bacilli show well marked segments. They retain Gram's stain, and show polar granules. On *serum* small round whitish colonies and on *agar* small round dry pearly-white colonies are formed. On *gelatin* there is very little growth, and on *potato* none. *Broth* does not become clouded but a slight granular deposit is formed. In *glucose broth* acid is produced. Entirely non-pathogenic to guinea-pigs.

Cerebro-spinal fluid.

Morrell and Wolf (1906) on two occasions obtained diphtheria bacilli during life from the cerebro-spinal fluid of a child suffering from general miliary tuberculosis and tubercular meningitis, but without any

signs of diphtheria. The organisms were typical in morphology and cultural reactions, and produced acid from glucose and dextrin, but not from saccharose or inulin. Inoculated guinea-pigs died in 48–56 hours with gelatinous oedema and supra-renal reddening.

Head and Wilson (1899) isolated after death typical virulent diphtheria bacilli from the ventricles and cerebro-spinal fluid of a woman suffering from suspected rabies.

Non-virulent diphtheria-like bacilli have been isolated from the brain and spinal fluids by Johnson and Goodall (1902) and Robertson, McRae and Jeffery (1903).

Diphtheria bacilli have in some instances been separated from the organs of persons dead of the disease (see Chapter III, p. 99), and diphtheria-like bacilli have been found in the sputum and organs of persons suffering from general paralysis of the insane (p. 441).

Kruse and Pasquale (1894) isolated a non-pathogenic organism, having the cultural and morphological peculiarities of the diphtheria bacillus, from the pus of a liver abscess following dysentery. They gave it the name of *Bacillus clavatus*.

Summary.

A very large number of bacilli have been obtained from the secretions of healthy persons and from various lesions of the throat, nose, eye, ear, skin, and genitalia, as well as from various animals and from milk, which more or less closely resemble the diphtheria bacillus in morphology. Many of them also closely resemble it in staining reactions and in cultural characters. The majority are non-pathogenic to laboratory animals, but some are pathogenic. Of the latter a few produce septicaemia, and others less acute conditions. All these bacilli may be roughly divided into groups according to the differences they exhibit in culture. The members of one group, though derived from widely different sources, closely resemble true diphtheria bacilli in all respects except in their action on animals. Amongst this group may be placed the non-virulent diphtheria bacillus, the bacilli described by Robertson, Kruse and Pasquale, and many others, which apparently possess no cultural characters by which they can be separated from the diphtheria bacillus.

Other groups are peculiar in producing coherent or adherent colonies on serum, extensive, often coloured, growths on potato, liquefaction of serum or gelatin, soluble pigments, or in developing spores.

Another group of diphtheroid organisms produces no acid in glucose broth. According to the writer's experience true virulent diphtheria bacilli never produce gas or possess any of the above characters, and descriptions of virulent diphtheria bacilli possessing any of them are rarely to be met with. Bacilli which show any of the above characters may therefore be separated from true diphtheria bacilli.

Many of the organisms described possess no pathological significance, and appear to be harmless saprophytes. Their presence may, however, lead to erroneous diagnoses, and unjustifiable conclusions as to the nature of the disease, the method of treatment, and the distribution of diphtheria bacilli. A few produce severe lesions in man, which may be mistaken for diphtheria. Judging from the experiments of Ruediger, Hamilton and Horton such organisms are not very uncommon and their importance has not yet been sufficiently recognised.

In considering the distribution of diphtheria bacilli amongst non-contacts and the diphtheritic origin of various lesions, the frequent occurrence of diphtheroid bacilli in many parts of the body must be borne in mind, and all such organisms thoroughly tested before any final decision is given[1]. Outside the human body diphtheroid organisms have also been found in milk, on the normal and diseased mucous membranes of many animals, and in other situations. In dealing with organisms found under these conditions similar precautions are necessary.

[1] See also pp. 264, 265.

CHAPTER X.

DIPHTHERIA-LIKE DISEASES.

Characters of diphtheritic lesions. Organisms which have been found associated with non-diphtheritic membranous lesions of the mouth, nose and larynx: Streptococci, Staphylococci, Brisou coccus, Pneumococcus, Bacillus of Friedländer, Bacillus coli, fusiform bacilli and Spirilla, Leptothrices, Yeasts, Syphilis.

BEFORE proceeding to describe the diseases which simulate diphtheria, it may be well to recapitulate briefly the various forms in which diphtheria may be met with in different parts of the body.

Diphtheria most commonly affects the posterior portions of the buccal cavity, producing in its typical form pseudo-membranes covering a part or the whole of the tonsils and the adjacent structures. Examples have, however, been encountered in which the membrane covered almost the whole of the surfaces within the buccal cavity, and on the other hand true diphtheria may show only very limited changes such as small membranous areas on the tonsils, white plugs in the crypts, or even simple redness on the tonsils without membrane. Diphtheritic membranes confined to the tongue have been noted by Trevelyan (1900), Thiercelin (1898), and Wharton (1895, bacilli virulent), and to the lips and gums by Trevelyan (1900) and Flexner and Pease (1895, two cases). Diphtheria bacilli have also been observed in noma by several observers (p. 348). Diphtheritic conditions of the larynx and trachea may also vary from extensive membranous formations to very slight lesions. Horne (1905), for example, mentions a case in which no diphtheria bacilli could be found during life, and were only obtained at the autopsy from the ventricle of the larynx.

In a similar manner diphtheria of the nose manifests itself in a variety of ways. Membranous or fibrinous rhinitis, acute or subacute catarrh, atrophic rhinitis in certain forms (Symes, 1903) and external rhinitis (Todd, 1898) may all be caused by virulent diphtheria bacilli.

Diphtheritic conjunctivitis has been reported by numerous observers, and virulent diphtheria bacilli have been encountered in ear discharges on several occasions. Membranous lesions of the external female genital organs, including the labia, vulva and vagina, and a few cases of lesions of the penis due to diphtheria bacilli have been recorded. Lesions of the skin in many parts of the body as well as abscesses and ulcers associated with the presence of virulent diphtheria bacilli have also been recorded. More rarely diphtheria bacilli have been observed to be present in lesions of the internal organs without external manifestations of the disease. Thus diphtheria bacilli have been cultivated from the brain in a supposed case of rabies by Head and Wilson (1899), from the cerebro-spinal fluid of a case of tuberculosis during life by Morrell and Wolf (1906), from the heart valves by Howard (1894), from a pneumonic lung by Ohlmacher (1895), etc.

The clinical manifestations of diphtheria are so divergent that the widest experience cannot hope to diagnose correctly on clinical grounds alone any but the more typical cases. Even in such cases mistakes are not infrequently made. The assistance of bacteriology is necessary before the identity of the less typical examples can be proved, and bacteriological methods should always be employed in the case of persons presenting throat or nose lesions, who have recently been in contact with diphtheria.

On the other hand a few examples do undoubtedly occur in which bacteriological evidence is at fault in the first examinations. In such cases the growth of the diphtheria bacilli in the culture tubes may be retarded or overwhelmed by the accompanying bacteria, or it may be inhibited by the presence of antiseptics, or from some other cause the bacilli may not develop. The first of these factors, however, seldom comes into action except in extremely foul ulcerative cases, and the proportion of erroneous bacteriological diagnoses from all causes is very small.

Even the anatomical condition of the membrane cannot be regarded as a certain proof of the nature of the disease. "As a rule the exudate of diphtheria is firmly incorporated with the underlying mucous membrane and cannot be removed without leaving a bleeding surface, at least until convalescence. The tissues surrounding the exudate are more or less inflamed and swollen. When other bacteria produce the irritant the exudate, except in the cases due to the bacillus described by Vincent, is usually loosely attached, collected in small

masses, and easily removable. Exceptions, however, occur in both these diseases, so that in true diphtheria, the exudate may be easily removed, and in lesions due to other bacteria the exudate may be firmly attached" (Park, 1900).

Organisms which have been found associated with Non-diphtheritic Membranous Lesions of the Mouth, Nose and Larynx.

Streptococci. Numerous observers have recorded cases of membranous inflammation of the mouth in which streptococci have been the only organisms present. Prudden (1889) studied a series of 22 fatal cases, and Woodhead (1901) found streptococci to be the only organisms present in 565 out of 1960 cases diagnosed on clinical grounds as diphtheria, in which no evidence of diphtheritic infection was obtained.

Staphylococci. Examples of membranous inflammation due to staphylococci are of very common occurrence, and even limited outbreaks of this disease have been described.

Mixed infection with streptococci and staphylococci is a more common cause of pseudo-diphtheritic lesions than pure infection with either of these organisms. Woodhead, for example, found that 985 (50·2 %) of his 1960 cases of pseudo-diphtheria were caused by mixed infection with these two organisms.

Brisou coccus. This organism has been often observed in pseudo-diphtheritic conditions, and forms colonies resembling those of the diphtheria bacillus. Its characters have been described by Roux and Yersin. Chaillou and Martin (1894) mention cases due to this organism in which the membrane extended into the trachea necessitating tracheotomy. Stone (1904) has recently described a peculiar diplococcus occurring in acute inflammatory conditions of the throat accompanied by a severe toxaemia. In many cases a distinct white tenacious false membrane is present on the tonsils. The adjacent sides of the cocci are flattened and they show distinct metachromatic granules when stained by Loeffler's method.

Pneumococcus. Cases of membranous inflammation of the fauces, occurring during the course of pneumonia, have frequently been observed, and many instances have also been recorded unaccompanied by pneumonia [Concetti (1893), Chaillou and Martin (1894), De Blasi and Russo-Travali (1896), Möller (1903), etc.]. Some examples of very extensive lesions are recorded, for example exudation covering the

tonsils, pillars of the fauces and uvula (Jaccoud, 1891), or the entire pharynx, palate, lips, and nasal cavities (Vedel, 1898). In both these instances pneumococci were the only organisms found, and in the latter case their virulence was proved. Perhaps the most remarkable example of extensive membrane formation by the pneumococcus is that described by Cary and Lyon (1901). During an attack of acute lobar pneumonia there occurred profuse pseudo-membranous exudations upon nearly all the mucous membranes of the body, tonsils, lips, gums, cheek, under surface of tongue, soft palate, fauces, nose, conjunctiva, anus, and glans penis. Diphtheria bacilli, streptococci, saccharomyces, etc., were all looked for, but were not found. Virulent pneumococci were proved to be the only organisms present in all the lesions. Another very interesting case was observed by Netter (1891). A boy aged three years suffering from varicella showed urgent symptoms of laryngitis requiring tracheotomy, through the wound of which a false membrane was expelled containing no diphtheria bacilli but only pneumococci. A similar case is also recorded by Seuvre (1898). In summarising his observations on the infections of the mucous membranes by pneumococcus Foulerton (1902, p. 293) makes the following remarks. "The exact bacteriological identification is rendered difficult by the fact that the organism is a frequent parasite of the healthy mouth. But the number of cases in which there are the formation of false membrane and destruction of the superficial layer of the epithelial membrane associated with the presence of the coccus and without any other of the bacteria —*B. diphtheriae, Streptococcus pyogenes, Saccharomyces albicans*— which are known to cause similar lesions, leaves little doubt as to the fairly frequent occurrence of an acute membranous pharyngitis due to this cause."

Bacillus of Friedländer. This organism has been occasionally found in cases resembling diphtheria (Michelazzi, 1904). Nicolle and Herbert (1897) found it in pure culture in six out of 1,600 cases of angina, and state that it is capable of producing chronic, subacute and acute anginas, sometimes accompanied by a continuous membrane.

Bacillus coli. Lemoine (1895) observed bacillus coli together with streptococci and pneumococci in false membranes, and Chaillou and Martin (1894) noted two cases in which liquefying organisms resembling bacillus coli were alone present.

Fusiform bacilli. Vincent (1896) was one of the first to call attention to the presence of fusiform bacilli and spirilla (Pl. XIV, fig. 4) in connection with ulcerative and membranous conditions in the mouth.

Consequently the pathological process associated with these organisms is frequently termed Vincent's angina. Since 1896 numerous observers[1] have noted their presence in various lesions, and even in the healthy mouth. These fusiform bacilli are of considerable interest since many of the conditions in which they are found closely simulate diphtheria, and the organisms themselves have to be carefully distinguished from diphtheria bacilli in preparations made directly from the throat. Although they have been so frequently detected in such preparations their isolation has been attended with difficulty, and appears only to have been successfully accomplished by three investigators, Veillon and Zuber (1898), Ellerman (1904) and Weaver and Tunnicliffe (1905). The following description is taken from the latter authors:

In smear preparations the bacilli are seen as long slender rods usually with pointed ends. Occasionally the ends are rounded, or the rod thicker, or it is slightly bent. Their length varies from 6—12 μ. They are generally scattered uniformly over the field but sometimes irregular clumps are seen. They may be stained by Loeffler's methylene blue, but are best stained with carbol fuchsin, and do not retain Gram's stain. They are non-motile and show no polar bodies. The bacilli may be grown in mixed culture in sugar-free broth, with or without the addition of ascites fluid. Pure cultures can be obtained by smearing the surface of horse-serum-agar slants and cultivating anaerobically at 37° C. for 3—5 days.

In pure culture the organisms are obligatory anaerobes. The colonies appear as delicate white discs which become confluent. A small moist growth is produced on Loeffler's serum, a delicate white confluent growth on ascites agar, a delicate cloud along the line of inoculation on agar, and a slight flocculent growth in sugar-free broth (Pl. XIV, fig. 5). There is no growth in milk, nutrient broth, or on potato. Cultures on media containing serum or ascites fluid give off a very foul odour.

On ascites agar, horse-serum-agar and Loeffler's medium they no longer appear as fusiform bacilli but as long delicate filaments with darker bodies at intervals (Pl. XIV, fig. 6). Veillon and Zuber (1898) produced small abscesses in rabbits and guinea-pigs by the inoculation of pure cultures, but Weaver and Tunnicliffe (1905) found that injections of pure cultures into the muscles or under the skin of rabbits and guinea-pigs were without result. In mixed cultures they produced abscesses.

Tunnicliffe's (1906) most recent investigations point to the conclusion that the fusiform bacilli and spirilla are not different organisms but merely different stages in the life history of the same organism. She describes the various appearances met with in cultures in the following words: "The organisms present the same morphological appearance in whatever media grown. They are extremely polymorphous, appearing as quite different organisms at different periods of their development. They are usually during the first 24 hours of their growth delicate pointed rods from 3 μ to 10 μ in length. As a rule they show deeply staining bodies

[1] Beitzke (1904) gives an excellent *résumé* of the literature.

or bands, most often two in number, not situated at the ends. The bacilli are usually straight, sometimes bent. The bacilli often strikingly resemble the barred forms of diphtheria bacilli. They are often slightly larger in the centre but not always. In the young cultures a few smaller, 1·5—4 μ in length, and plumper bacilli are also sometimes found. They have very thick unstained bodies, with deeply stained rounded ends. The swollen bodies resemble spores, but do not stain as such. Both forms appear in pairs, end to end, at obtuse angles, and in rows. In some of the longer bacilli, usually during the first days of their growth, a few spores are seen. There is usually one in a bacillus, but occasionally there are two. They are situated either at one extremity or near the centre. They may be seen within, or partly without, or entirely outside the bacillus. The development from the round spores into the very short plump bacilli with dark extremities may be observed in a hanging block. In 24 hours or even later, filaments of various lengths are formed. Some of these filaments are of the same diameter throughout and contain as a rule deeply staining bodies, sometimes round, oftener like bands. Similar ribbon shaped forms are frequently seen in smear preparations from the gums. Some of the filaments stain uniformly. As a rule many of the filaments are seen to be made up of strings of bacilli, which are joined together at the dark bodies. The bacilli forming the filaments vary in size and shape as the bacilli in the earlier cultures do. The filaments are sometimes straight, sometimes wavy. Involution forms in a great variety of shapes are frequently observed. In the older cultures the filaments often stain irregularly. Clear spaces resembling vacuoles are often seen. They are simply the unstained bodies of the short, plump bacilli which in chains are so close together as to appear as vacuoles.

"Soon after or simultaneous with the appearance of the filaments, most often on the fourth or fifth day, spirals are observed, sometimes in enormous numbers. As a rule they stain uniformly; others show the dark bodies seen in the short bacilli and filaments. Often it is easily seen that the spirilla are made up of chains of short bacilli similar to the straight filaments. The spirals are sometimes in the form of corkscrews, more often the turns are not so sharp, nor so deep. They form one to twenty curves. The turns are sometimes rounded, sometimes very pointed. The pointed ones are especially marked when the spirilla can be seen to be made up of bacilli and when there is only one bend. Some of the larger spirals extend across the whole field, more often they are shorter, showing four or five turns. These shorter forms are from 4—5 μ in length. They vary considerably in the depth of the curve as do the uncultivated spirilla. The ends are usually pointed. Toward the extremities the curves sometimes become more and more broad. Involution forms are seen in the spirilla as well as in the filaments. In some of the cultures the spirilla alone are found, but usually the filaments and short bacilli are also present. By the 10th to the 15th day fewer filaments and spirilla are seen, but as a rule, even in the older cultures (55 days), spirilla can still be found. Both the bacilli and spirilla stain by methylene blue, gentian-violet, Giemsa, Romanowsky, carbol-fuchsin, and carbol-gentian violet."

Cultures of all ages inoculated into guinea-pigs produce no result.

Leptothrix. Meunier and Bertherand (1898) describe a case in which a diphtheria-like membrane was found. A leptothrix was

present in great abundance on the tonsils, uvula, and soft palate. Sections of the membrane showed large numbers of these organisms.

Yeasts. Pseudo-membranous lesions are occasionally met with, in which yeasts, usually *Saccharomyces albicans*, are the most abundant, or the only organisms to be found.

Syphilis. Campbell (1904) and others have called attention to the resemblance to diphtheria of chancre of the tonsil. The chancre itself may have a pseudo-membranous appearance and the accompanying general phenomena and enlarged glands add to the difficulty of diagnosis. Even an intranasal chancre has been mistaken for nasal diphtheria (Rolleston, 1906).

CHAPTER XI.

PREVENTIVE MEASURES.

General preventive measures. Notification. Isolation. Disinfection. Source of infection. Closure of schools. *Special preventive measures.* Primary and secondary examinations of clinical cases and contacts. Classes of persons who ought to be examined. Isolation of mild cases and infected contacts. Infected families. Schools. Isolation homes. Hospitals and institutions. Period of isolation. Causes of misleading negative cultures. Proportion of negative followed by positive cultures. Persistence of diphtheria bacilli in the throat and nose. Attempts to hasten the disappearance of the bacilli. Results of isolation of convalescents, mild cases, and contacts in families, schools, institutions, hospital wards, and towns during outbreaks. The prophylactic injection of antitoxin. Summary.

Preventive Measures.

THE measures which may be taken to check the spread of diphtheria will be most conveniently discussed under two headings, namely (1) general and (2) special measures. Under the heading of general preventive measures are considered those means of prevention which are applicable in the case of any other severe infectious disease, and the special modifications which are necesssary in the case of diphtheria, whether they apply to the individual, or to collections of individuals, or food substances. The application of bacteriology in checking the spread of the disease will be discussed under special preventive measures.

I. General Preventive Measures.

Notification. Persons suffering from diphtheria are required by law to be notified to the Medical Officer of Health. In the case of persons showing the typical symptoms of diphtheria no difficulty arises. The fact that the disease may manifest itself in a variety of ways and that

during outbreaks many persons may be encountered who are carrying virulent bacilli, without any signs of ill-health, complicates the question of notification. Whether persons suffering from atypical forms of the disease and healthy infected contacts should be notified are points which have frequently been discussed. Most authorities agree that the former class should be notified as suffering from diphtheria, and treated in the same manner as those who show typical clinical symptoms. On the other hand the general opinion is that healthy infected contacts should not be notified, but should be dealt with separately from those who show clinical signs. A few authorities, however, consider that even these persons ought to be notified. This question was very carefully considered by Cobbett (1901) in dealing with his first outbreak at Cambridge, and he came to the conclusion that the notification of such persons was undesirable for many reasons.

Diphtheria is a disease, and therefore a person cannot be held to have diphtheria who remains perfectly well, and notification is resented on this ground.

"It has been pointed out that without notification the Medical Officer of Health has no power to deal with these persons, but that armed with this instrument he can compel them to be isolated. In answer to this, it may be said that he could only compel the removal of those for whom it could be shown that isolation was impossible at home, and that too on the receipt of an order from a magistrate." Further, compulsory isolation following on notification would raise general opposition to all measures attempting to deal with these persons, and since persuasion has in most cases been perfectly satisfactory it is undesirable to create opposition by resorting to forcible measures.

The fact is that nothing can be done unless the people back up the measures. When it is a case of dealing with public schools, and the class of persons who send their sons to them, there is little or no difficulty in carrying out the bacteriological examination of contacts, and the parents see to the isolation of their infected children, for they at once recognise that the measures proposed are for their own interest. The poorer class will take the same view if the matter is fairly explained to them. We must therefore in such matters act by persuasion rather than by force. If persuasion fails, the education authorities have the power of refusing to allow the infected scholars to return to school until they have been officially certified to be free from diphtheria bacilli.

Isolation. Isolation hospitals[1] are provided by the Local Authorities in which patients suffering from diphtheria, who cannot be efficiently isolated in their own homes, may be treated. In whatever way patients are isolated it is highly desirable that the isolation should be efficiently carried out so that the possibility of the spread of the disease by contact should be reduced to a minimum. In all cases the methods by which the disease is conveyed from person to person should be explained to the attendants, and precautions taken to prevent its dissemination by infected objects.

Disinfection. The various examples which have been quoted of the finding of diphtheria bacilli on infected articles and in dust show the necessity for sterilising, whenever possible, all articles and toys which have been used by the patients immediately before and after the development of clinical signs. Although the probability of infection by means of dust, etc., is very small, it is generally customary to disinfect the room occupied by the patient after removal to the hospital, or after the termination of isolation, if the patient has been kept at home.

During the progress of the disease all cups, plates, etc., used by the patient ought to be sterilised by boiling after use, and all soiled linen and other similar articles ought to be sterilised by some efficient means. Articles of little value and dressings should be burnt.

The importance of these precautions, which relate more particularly to the spread of the disease to family or very close contacts, is widely acknowledged, and once the disease has been recognised more or less efficient precautionary measures are usually adopted.

Comparatively little attention has, however, been paid to similar precautions for checking the spread of diphtheria bacilli amongst school children. It has already been shown that the bacilli are most frequently communicated by means of articles such as pencils, which pass from mouth to mouth. Suggestions have frequently been made, that each child should possess its own slate, pens, pencils, etc., and that these articles should be frequently and systematically sterilised when diphtheria is prevalent. In institutions where the children receive one or all their meals, the same precautions ought to be taken in regard to knives, forks, cups, etc.

Source of infection. Once diphtheria has broken out it becomes the duty of the authorities to trace as far as possible the origin of the infection, so as to check any further spread from that source.

[1] The provision of Isolation Homes for infected contacts belongs more appropriately to the Bacteriological measures and is discussed later (p. 405).

Since the source of most infections is personal contact, inquiries ought to be first directed to the possibility of infection having occurred in this way, and a bacteriological examination made of the members of the patient's family and any persons whom the inquiry may indicate to have been possibly instrumental in carrying the disease. By this means it is frequently possible to detect mild unrecognised cases which act as carriers (p. 309). Bacteriological examinations ought also to be made of persons who are likely to have been infected by the patient, and may be capable of transmitting the disease. The classes of persons whom it is desirable to examine under these conditions are fully considered later (p. 401).

If no source of personal contact is discovered, and especially if cases arise apparently independently of one another, suspicion is directed to the milk supply. Here again the bacteriological examination of those who are connected with the cows, the dairy, and the distribution of the milk is likely to be of more service than the examination of the milk itself, since the isolation of the diphtheria bacillus from milk is a difficult matter, and has only been successfully accomplished on three occasions. Still more rarely has it been definitely proved that the cows themselves are the source of the disease (p. 285). Suspicion has frequently been directed to other domestic animals and birds as the source of the disease, but bacteriologically the suspicion has very seldom been confirmed (p. 304). Infected articles used by many persons, such as speaking tubes, and the cups of public fountains, etc., may occasionally play some part in the dissemination of the disease, and ought not to be neglected. The list, which has been given, practically exhausts all the means by which the disease has been proved to have been propagated, since no trustworthy evidence exists that it may arise through the agency of putrefying refuse, sewer gas, polluted soil, or unhealthy surroundings. By their effect on the general health these agencies may, however, render those who are exposed to their influence more susceptible to the disease.

Schools. The influence of schools and institutions, in which large numbers of individuals of susceptible age are brought together, is a subject which has received great attention. It seems to be now generally held that such places play an important part in the dissemination of the disease through the presence of mild unrecognised cases and infected contacts, who readily pass on the bacilli which they have acquired owing to the habits which prevail amongst the children (p. 318).

In serious outbreaks it has generally been the custom to close the schools with the object of preventing the daily meeting of large numbers of children. No doubt to some extent this proceeding succeeds in its object, but it has the disadvantage of rendering the examination of the scholars more difficult, and the probability of discovering most of the carriers of the bacilli more remote. Further, while the children are attending the schools cases of mild sore throat and other ailments are brought to the notice of the authorities and can be immediately examined, whereas while the children are at home such slight ailments are liable to pass unnoticed. If such cases are found on bacteriological examination to be suffering from mild diphtheria, their immediate contacts can be easily singled out for examination. It is therefore desirable, whenever possible, to bacteriologically examine the school children before the closing of the schools, and thereby take the opportunity of dealing with the infected children who may be discovered. The methods which have been employed in dealing with these persons and their results are given in the next section. The question of dealing with the public elementary schools is complicated by the fact that the children from the infected schools are liable to mix on Sundays with the children from non-infected schools in the Sunday Schools. This intermingling of the scholars from healthy and infected day schools is probably a frequent cause of the extension of the disease to schools which up to that time have remained uninfected. The closing therefore of Sunday Schools is one of the first measures which ought to be enforced when diphtheria becomes prevalent.

II. Special Preventive Measures depending on Bacteriological Methods.

More is definitely known about the transmission of diphtheritic infection than of any other epidemic disease. Owing to this fact and the ease with which most suspected exudates and materials can be examined, diphtheria readily lends itself to bacteriological methods of prevention. In this section the various bacteriological preventive measures which have been used, and the measures which depend on them, together with the results which have been obtained, are discussed.

The bacteriological measures may be considered under the following headings:—

(1) The primary examinations of clinical cases and the subsequent examinations of convalescents. (2) The primary examinations of contacts

and the subsequent examinations of those who are found to be infected. (3) The classes of contacts who should be examined in connection with cases of diphtheria. (4) The isolation of infected contacts. (5) The time during which convalescents and infected contacts should be isolated. (6) Records relating to the persistence of diphtheria bacilli in the throat and nose, etc. (7) Attempts to hasten the disappearance of the bacilli. (8) The results of isolating convalescents and infected contacts until the disappearance of the diphtheria bacilli in (*a*) families, (*b*) schools, (*c*) institutions and hospital wards, and (*d*) towns. (9) The prophylactic injection of antitoxin.

(1) *The primary examinations of clinical cases and the subsequent examinations of convalescents, and* (2) *the primary examinations of contacts and subsequent examinations of those who are found to be infected.*

The methods of practical bacteriological examination in all these cases have already been discussed (p. 340) at some length, but a statement of the principal points may be desirable. The bacteriological diagnosis in most clinical cases is easy, but in all instances in which doubtful bacilli are found it is the duty of the bacteriologist to test and prove the correctness of his opinions by every means in his power. When the patient is becoming convalescent the bacilli may be present in large numbers or their numbers may be very small. In the latter case especial care should be taken to obtain satisfactory swabs, at least 12 hours after the application of any antiseptic substances.

Should no diphtheria bacilli be encountered at the first examination the cultures should be recultivated for a period of 24 hours and again examined. When the bacilli has disappeared from the seat of the original lesion, the other situations in which they are liable to lurk should also be examined, in order that the patient should not return to the community still harbouring virulent diphtheria bacilli.

In the case of infected contacts the bacilli may or may not be abundant from the start. It is therefore desirable in examining contacts to subject all negative cultures to a further period of incubation and examination. The same precaution ought to be observed in all the subsequent examinations of infected contacts.

(3) *The classes of persons who ought to be examined in connection with cases of diphtheria.*

Reference to the section on Infection by Contact shows that the proportion of infected contacts is highest amongst the members of the

family of the patient, next highest amongst close contacts in hospital wards and institutions and lowest amongst the more remote contacts in schools. A careful examination of the results of the observations on wards, institutions and schools reveals the fact that in these cases the proportion of infected contacts is low, because many are examined who have had no direct relationship with the case. It is those who have been in attendance on the patient, or have been situated near him either in wards or in schools, who are most commonly found to be infected. Illustrations of these facts have already been given (pp. 309—318).

It therefore follows that in connection with a case of diphtheria the members of the family, and all who have been in close connection with him at home, should be immediately examined, as well as his more intimate friends, and at least certain members of his class who sat near him at school. These proceedings not infrequently reveal mild unrecognised cases and healthy infected contacts, especially in connection with those instances in which the clinical signs are at first mild, and only after some days become sufficiently severe to be recognised. The discovery of unrecognised cases has not only frequently led to the prevention of further transmission of the disease, but has afforded valuable information as to its origin.

When a serious outbreak has to be dealt with in a school, it is no longer possible to hope that these measures will be sufficient to trace all the infected persons. It then becomes desirable to examine all the members of the class to which patients belong, or even the whole school.

When an outbreak of diphtheria occurs in a hospital ward, the throats and noses of all attendants as well as of the occupants of the ward, especially those who are closely connected with the patients either by occupying adjacent beds or by visiting them, should be examined. In epidemic times outbreaks of diphtheria in the wards of general hospitals may be prevented by systematically examining all the children who are admitted.

The same procedure has met with considerable success in preventing the introduction of diphtheria into Scarlet Fever wards (Chapter XII). In all these cases special attention should be directed to persons who show any abnormal condition of the fauces or nose, or who are suffering from ear discharges.

When there is reason to suspect the milk supply as the cause of the outbreak not only should the same precautions be taken as in other outbreaks, but a thorough examination should be made of all persons

who are connected with the milking of the cows, and with the dairy, and their families. When the distribution of the disease points to the infection of the milk during delivery, it would also be desirable to examine any persons who had access to the milk after it left the dairy along the infected route.

(4) *The isolation of mild cases and infected contacts.*

Almost all authorities have advocated the isolation of persons, who are found to be suffering from abnormal conditions of the throat or nose associated with the presence of diphtheria bacilli. On the other hand while some have advocated the isolation, whenever possible, of all infected contacts, even those who show no manifestations of disease, others think, that while it is theoretically desirable to do so, this proceeding is impracticable except in those schools in which the scholars reside on the premises, hospital wards and certain institutions. Others again appear to believe that the danger of infection from such persons is so slight that measures to insure their isolation are, in the majority of cases, unnecessary. Some of those who take this latter view are not averse to the isolation of infected contacts, when isolation can be easily accomplished.

Reference to the beneficial results which have followed on the isolation of healthy infected contacts under all conditions, indicates the desirability of isolating infected contacts, whenever this can be accomplished (see p. 424).

Infected Families.

When an infected contact is discovered amongst the children of an infected family belonging to the well-to-do class, it is frequently sufficient to explain to the parents the danger which the other members of the family run from the presence of this child amongst them. Arrangements can then usually be made for the isolation of this child in some room, until the bacilli have disappeared, and for the adequate disinfection of the articles used by him. A person who is not in the habit of mingling with the healthy members of the family should, if possible, attend on the infected child. Amongst the poorer classes such an arrangement is usually impossible both from lack of accommodation and from the fact that no one can usually be spared to see that efficient isolation is maintained. It is also generally extremely difficult to make

the parents understand that all danger has not passed within a few days.

Consequently in such cases home isolation is a mere farce, and an attempt should be made to obtain the removal of the child to an isolation home, or some place where it can not only be kept from contact with other susceptible individuals, but treated with antiseptics in order if possible to hasten the disappearance of the bacilli.

A more serious question arises when it is found that one of the adult members of the family is harbouring diphtheria bacilli. In the well-to-do classes there is little difficulty in making some satisfactory arrangement whereby the probability of the younger susceptible members becoming infected is very greatly diminished. Amongst the poorer classes, however, more difficulty is experienced in deciding how to treat these persons. On the one hand the infected person may be a source of danger to other adults under certain conditions. There is, for example, a grave risk of spreading the disease when the infected person is employed in a factory, shop, or other place of business where the employees are constantly in close contact or have their meals together. On the other hand the children of the family are liable to become infected from this individual, and convey the disease to other children. Under these circumstances the employer is sometimes willing to allow such a person to stay away until he is free from diphtheria bacilli. Unless an arrangement of this description can be made, the infected individual cannot be compulsorily isolated and the family deprived of their means of livelihood. Cobbett (1901, footnote, p. 488) met with this difficulty and decided that "it was not expedient, as a rule, to examine the parents or bread winners, on account of the impossibility of isolating them without provision being made for the support of those dependent on them."

Wesbrook (1900) had previously suggested in his regulations for quarantine in diphtheria that wage-earning members of the families should be exempted from isolation as long as they did not remain in contact with the infected members.

If it is decided that it is not desirable to examine parents and bread-winners, the risk of some of them being infected and spreading the disease has to be borne in mind in investigating the source of infection in fresh cases. Two instances illustrating the conveyance of the disease by infected parents have recently come under the writer's notice. In each instance one of the children developed clinical diphtheria, and the other children of the family were examined and were found to be free from

diphtheria bacilli. In one family two cases and in the other one case arose between four and five weeks later. Again on examination the other children were found to be free, but in one family the mother, and in the other the father, were found to be harbouring virulent diphtheria bacilli. The former had also given the infection to a neighbour's child, which she had nursed when it was suffering from the effects of a slight accident.

Schools.

In schools, whether large or small, attended by the children of the well-to-do classes the isolation of mild cases and infected contacts can generally be easily arranged in some of the buildings or rooms of these establishments.

The isolation of such children when found among the scholars of the public elementary schools is, however, a more difficult matter. As has been pointed out the mere exclusion of these children from the schools and the attempt to isolate them efficiently in their homes are somewhat unsatisfactory, although considerable benefit has been derived from these measures in some instances. It also seldom happens that there are any suitable rooms or buildings connected with such schools which can be used for the purpose. If, therefore, any attempt is made to check the spread of the disease through infected contacts, suitable isolation homes ought to be provided for them. In epidemic times a suitable house in some more or less isolated position can usually be procured and used as an Isolation Home under the direction of properly qualified nurses. Such Isolation Homes have been provided at Cambridge (1901–4) and Colchester (1901).

Isolation Homes.

In every instance in which any form of Isolation Home has been used both healthy infected contacts and mild cases of diphtheria have been admitted at the same time.

This association of healthy persons with cases of diphtheria cannot be otherwise than dangerous, unless the bacteriologist can be relied upon to exclude all but those who are harbouring the true diphtheria bacillus. Healthy individuals harbouring the virulent diphtheria bacillus are not likely to be harmed by receiving diphtheria bacilli from others. This argument does not, however, necessarily apply to those persons who are found to be harbouring the non-virulent diphtheria bacillus. It may be questioned whether it is necessary to isolate these

persons, and whether, if isolated amongst those who carry about the virulent diphtheria bacillus, they are liable to catch diphtheria. In practice, however, the virulence of the bacillus is only determined after isolation has been carried out. The probability of the interchange of bacilli amongst the isolated persons may be almost completely abolished by the frequent use of antiseptics, and by setting apart particular knives, cups, etc. which can be frequently sterilised, for the use of each inmate.

Under these conditions the experiences of Cobbett (1901) and Graham-Smith (1902–4) show that no ill effects are produced by the isolation of all these classes of persons in one place. The former (p. 491) records some very interesting observations on this subject. Five children, harbouring non-virulent diphtheria bacilli, lived in close contact for some time in a Home with twelve others, who were infected with diphtheria bacilli known to be virulent in the case of eight. This action was followed by no bad results, no case of diphtheria or even sore throat occurring amongst the healthy persons in the Home.

"From seven of the persons isolated in this Home, the bacilli were twice or oftener isolated and tested for virulence on the guinea-pig. The result was striking. Those admitted with a non-virulent diphtheria bacillus were never found to have acquired a virulent bacillus during their stay in the Home, nor was a non-virulent diphtheria bacillus ever found in a child in whom virulent bacilli had once been found. In the case of E. J. the bacilli (non-virulent) were isolated and tested 10 times in the course of the 15 weeks she remained in the Home. During five of these weeks her little sister V. J. was with her constantly and on three occasions the bacilli were isolated from her and proved fully virulent. Moreover from another girl, G. B., who remained in the Home almost as long as E. J., diphtheria bacilli were isolated and proved virulent no less than six times." The same evidence, but less strong, is afforded by the rest of the seven cases mentioned above.

Hospitals and Institutions.

Hospitals, Asylums and various Public Institutions generally afford ample facilities for the isolation of infected persons.

(5) *The period during which isolation of convalescents and infected contacts ought to be maintained.*

Even up to the present time very diverse views are held as to the time during which convalescents ought to be isolated. Judging from

certain reports there are some local authorities who allow diphtheria patients to return to their homes almost as soon as the clinical manifestations of the disease have disappeared, while others fix an arbitrary time limit from the date of the disappearance of the last symptoms. The time most commonly chosen is about four weeks. It is, however, now becoming very generally recognised, that such arbitrary time limits, although they may cover the period of infectiousness of the majority of patients, allow the return to the community of many individuals who are still capable of transmitting the disease.

The only means by which the patients can be proved to be no longer dangerous is the bacteriological test. The period of isolation or quarantine should therefore in all cases be regulated by the bacteriological findings. Amongst those who advocate this means of regulating the period of isolation, differences of opinion still exist as to the significance of the organisms encountered and the importance which should be attached to them. While some consider that isolation should be continued until the disappearance of the morphologically typical diphtheria bacilli, others think that even the typical bacilli lose their virulence during a prolonged stay in the throat or nose, and that after a time their presence in small numbers is no longer of much importance. Numerous investigations have already been quoted (p. 236) which show that diphtheria bacilli may retain full virulence for very long periods, and consequently without animal experiments there is no ground in any given case for considering that their virulence has diminished and that their presence may be neglected. Others again hold that diphtheria bacilli during convalescence become converted into Hofmann's bacilli, which are capable under unknown conditions of regaining their pathogenicity.

If the views of the latter group were followed patients would have to be isolated until the disappearance of all diphtheria-like bacilli. Figures, which have been already quoted (p. 205), show how commonly Hofmann's bacilli are found in the secretions of normal persons, and reference to many cases given in the following tables (pp. 408—413) shows that these organisms may persist for an indefinite time in the mouths of convalescents and healthy contacts.

Further it has been shown that there are many reasons for believing that the Hofmann's bacillus and other diphtheroid organisms are distinct species, having no connection with the diphtheria bacillus (Chapter VI). It is, moreover, impracticable to isolate many individuals until the disappearance of such organisms. In practice, therefore, this method cannot be adopted.

Table showing the virulence of diphtheria bacilli isolated from 112 *persons, clinical cases and contacts, out of* 115 *persons found to be harbouring diphtheria bacilli during an epidemic at Cambridge (Graham-Smith,* 1904).

(a) = Notified case. Diphtheria bacilli found.
(b) = "Contact" infected with diphtheria bacilli.
Δ = Virulent diphtheria bacillus.
Λ = Non-virulent diphtheria bacillus.
O = Diphtheria bacillus not found.
* = Hofmann's pseudo-diphtheria bacillus found.
SS = Small swelling in subcutaneous tissue of guinea-pig.
MS = Moderate ,, ,, ,, ,,
LS = Large ,, ,, ,, ,,
+ = Death of guinea-pig.

N = No result. Guinea-pig not affected.
dark red = Suprarenals very dark in colour.
red = Suprarenals deep red in colour.
pink = Suprarenals pink in colour.
present = Pseudo-diphtheria bacilli found on one or more examinations.
0 = ,, ,, ,, not found at any ,, (last column).

In I, II, III or IV days, *i.e.* within 24, 48, 72 or 96 hours.

No.	Initials	Results of consecutive examinations	Days during which diphtheria bacilli remained in throat	Reaction in glucose broth	Animal inoculations						Results of Autopsy			Pseudo-diphtheria bacillus
					Wt. of guinea-pig in grammes	Dose of 48 hours broth culture in c.c.	Results. Days I	II	III	IV	Oedema	Suprarenals	Fluid in pleura	
St Matthew's School (Girls)														
1	A.S. (a)	O.O.Δ.Δ.Δ.Δ.O * * *	72	acid	170	·2	MS	+			extensive haemorrhagic	dark red	trace	present
2	W.B. (a)	Δ.O.O.O	8	,,	170	·2	SS	+			extensive	red	-	0
3	G.G. (a)	Fatal. No examinations												
4	K.M. (b)	Λ.O.O.O * * *	8	acid	180	·2	N	N	N	N (14th day)	-	-	-	present
					200	2·0	N	N	N	N	-	-	-	
5	G.C. (b)	Δ.Δ.Δ.Δ.O.Δ.O.Δ.Δ.Δ.Δ.O.O. O.Δ.O.O.O	44	,,	240	·2	SS	+			slight	dark red	-	0
6	W.L. (b)	Δ.O.O.O.O * * * *	8	,,	210	·1	LS	+			extensive	,,	·8 c.c.	present
7	E.P. (b)	Δ.O.O.O * * * *	8	,,	235	·1	MS	+			,,	,,	3·2 c.c.	present
8	C.S. (b)	Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.O.O.O * * *	29	,,	180	·2	MS	LS	+		very extensive	,,	-	present
9	R.S. (b)	Δ.Δ.Δ.O.Δ.O.O.Δ.O.O	83	,,	280	·1	MS	+			extensive haemorrhagic	,,	trace	0
St Matthew's School (Infants)														
10	H.O. (a)	Δ.O.O.O * * * *	21	,,	not tested						-	-	-	present
11	F.A. (a)	Δ.O.O.O * * *	17	,,	230	·1	MS	+			extensive	dark red	4·6 c.c.	present
12	F.E. (a)	Δ.O.Δ.O.Δ.O.O.O.O.O * *	63	,,	160	·2	SS	+			extensive haemorrhagic	,,	-	present
13	F.H. (a)	Δ. Fatal	-	,,	170	·2	MS	LS	+		extensive	,,	-	-

14	M.F. (a)	Δ.0.0.0	28	acid	190	·2	MS	+			extensive	dark red	·9 c.c.	present
15	N.W. (a)	Δ.Δ.Δ.Δ.0.0.0	24	,,	430	·3	SS	+			extensive haemorrhagic	,,	-	present
16	D.H. (b)	Δ.Δ.Δ.0.0.Δ.Δ.0.0.0.0	29	,,	225	·1	MS	LS	LS	+	,,	red	3·4 c.c.	present
17	D.E. (b)	Δ.Δ.0.0.0.0	14	,,	225	·1	SS	+			,,	,,	trace	present
18	V.L. (b)	Δ.0.0.Δ.Δ.Δ.Δ.0.Δ.Δ.Δ.0.0.0	41	,,	270	·1	MS	LS	+		,,	dark red	·4 c.c.	present
19	M.P. (b)	Δ.Δ.Δ.Δ.0.0.0	20	,,	240	·1	SS	+			extensive	,,	1·0 c.c.	present
20	T.E. (b)	Δ.Δ.Δ.Δ.0.0.0	22	,,	200	·1	SS	SS	+		slight	red	-	0
21	H.W. (b)	Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.0.Λ.0.0.Λ.Λ. Λ.0.Λ.0.0.0	52	,,	200 +200	·2 2·0	N SS	N N	N N	14th day N N	- -	- -	- -	present
22	B.T. (b)	Δ.0.0.0	9	,,	235	·2	LS	+			extensive haemorrhagic	dark red	2·3 c.c.	present
23	S.C. (b)	Δ.Δ.Δ.Δ.Δ.Δ.Δ.0.Δ.0.Δ.Δ.0. 0.Δ.0.Δ.0.0.0	51	,,	300	·1	MS	LS	LS	+	extensive	,,	-	present
24	G.T. (a)	Δ.0.0.0	17	,,	150	·2	MS	+			extensive haemorrhagic	red	-	present
25	S.M. (b)	Δ.0.0.0	9	,,	150	·1	SS	+			extensive	dark red	-	present
26	F.S. (b)	Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.0.Λ.0. Λ.Λ.Λ.Λ.Λ.Λ.Λ.0.Λ.Λ.0.Λ.Λ. Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ. Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.0.Λ	122	,,	180 ‡210	·2 2·0	N N	N N	N N	14th day N N	- -	- -	- -	0
27	W.O. (b)	Λ.0.0.0	9	,,	200	·2	N	N	N	14th day N	-	-	-	present
28	A.M. (b)	Λ	-	,,	340	2·0	N	N	N	14th day N	-	-	-	-
29	L.R. (a)	0.Δ.Δ.0.0.0	30	,,	170	·2	MS	+			slight	dark red	-	present
30	M.H (a)	Δ. Fatal	-	,,	410	·2	SS	MS	+		extensive haemorrhagic	,,	2·5 c.c.	-
31	J.M. (a)	Δ.Δ.Δ.Δ.Δ.Δ.Δ.0.0.Δ.0.Δ.0. 0.0.0.0.0.Δ.0.0	58	,,	210	·2	SS	+			moderate	,,	-	0
32	A.M. (b)	Δ.Δ.0.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ. Δ.0.Δ.Δ.0.Δ	47	,,	250	·2	SS	+			extensive	,,	12·0 c.c.	present
33	W.R. (a)	Δ.0.Δ.0.0.0.0	32	,,	225	·2	LS	+			,,	,,	1·0 c.c.	present

† Bacilli from 7th examination.

‡ Bacilli from 28th examination.

No.	Initials	Results of consecutive examinations	Days during which diphtheria bacilli remained in throat	Reaction in glucose broth	Animal inoculations						Results of Autopsy			Pseudo-diphtheria bacillus
					Wt. of guinea-pig in grammes	Dose of 48 hours broth culture in c.c.	Results. Days I	II	III	IV	Oedema	Supra-renals	Fluid in pleura	
34	C.L. (a)	Δ. Fatal	-	acid	195	·2	MS	+			extensive	dark red	3·2 c.c.	-
35	F.B. (a)	Δ.O.Δ.Δ.Δ.Δ.O.O.O.O.O	27	,,	210	·2	SS	MS	+		,,	,,	-	0
36	A.C. (a)	Δ. Fatal	-	,,	220	·2	MS	+			,,	red	6·3 c.c.	-
37	R.G. (a)	Δ.Δ.Δ.O.O.O.O.O.	25	,,	150	·2	SS	+			,,	dark red	2·0 c.c.	0
38	J.G. (a)	Δ.O.O.O.O.O	6	,,	190	·2	LS	+			,,	,,	4·0 c.c.	0
Sturton Street School														
39	A.L. (a)	Δ.O.O.O	14	,,	260	·1	SS	MS	+		,,	,,	1·3 c.c.	present
40	G.S. (b)	Δ.Δ.Δ.O.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ. Δ.Δ.Δ.Δ.O.O.Δ.Δ.O.Δ.O.O.O	27	,,	260	·3	LS	LS	+		extensive haemorrhagic	,,	6·3 c.c.	present
41	J.T. (a)	Δ.Δ.Δ.O.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ. Δ.O.Δ.Δ.Δ.O.O.O	58	,,	210	·2	MS	+			extensive	,,	5·5 c.c.	present
42	B.T. (b)	Λ.Λ.Λ.O.O.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ. Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.O.Λ. O.O.O	62	,,	190 †310	·2 2·0	N N	N N	N N	14th day N N	- -	- -	- -	present
43	M.E. (a)	Δ.O.Δ.O.O.Δ.O.Δ.O.Δ.Δ.O.O. O.O.O.O	32	,,	270	·2	LS	LS	+		extensive haemorrhagic	dark red	4·5 c.c.	0
44	S.E. (a)	Δ.Δ.O.O.O.O	22	,,	200	·2	MS	+			extensive	,,	·5 c.c.	present
Hospital														
45	F.H. (a)	Δ.Δ.O.O	18	,,	200	·2	SS	+			moderate	,,	-	0
46	C.B. (a)	Δ.O.O.O.O	15	,,	200	·2	MS	+			extensive	,,	-	0
47	M.S. (a)	Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.O.O.O	56	,,	220	·2	SS	LS	+		,,	,,	3·5 c.c.	0
48	R.C. (a)	Δ.O.Δ.Δ.Δ.Δ.O.O.Δ.O.O	41	,,	275	·2	MS	+			extensive haemorrhagic	red	4·8 c.c.	present

† Bacilli from 19th examination.

										14th day				
49	A.R. (a)	Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.0.0.0	51	acid	250	·2	N	N	N	N	-	-	-	0
50	H.R. (a)	Δ.Δ.Δ.0.0.Δ.0.0.0.Δ.0.Δ.Δ. Δ.Δ.Δ.0.0	99	,,	250	·2	SS	MS	+		extensive	dark red	4·5 c.c.	present
51	G.R. (b)	Δ.Δ.Δ.Δ.0.Δ.Δ.0.0.0.0	48	,,	250	·2	LS	LS	+		,,	,,	-	0
Sanatorium														
52	A.W. (a)	Δ.Δ	-	,,	250	·2	MS	+			extensive	,,	-	0
53	T.S. (b)	Δ.Δ.Δ.Δ.0	44	,,	200	·2	SS	MS	+		,,	,,	-	0
54	R.F. (a)	Δ.0.0.0	33	,,	210	·2	MS	+			,,	,,	9·0 c.c.	present
55	D.W. (b)	Δ. Fatal	-	,,	250	·2	MS	LS	+		,,	,,	5·6 c.c.	-
56	E.H. (b)	Δ.0.0.0	12	,,	320	·2	MS	LS	+		,,	,,	3·4 c.c.	0
57	B.M. (b)	Δ.0.0.0	13	,,	320	·2	LS	+			extensive haemorrhagic	,,	-	0
58	H.H. (b)	Δ.Δ.0.0.0.0	12	,,	350	·2	LS	LS	+		extensive	,,	-	0
59	E.H. (b)	Δ.0.Δ.0.0.0	10	,,	330	·2	LS	LS	+		,,	,,	-	0
60	L.B. (a)	Δ.Δ.0.0.0	25	,,	320	·2	SS	+			slight	red	7·5 c.c.	0
St Matthew's School (Infants—Autumn)														
61	F.B. (a)	Δ. Fatal	-	,,	300	·3	SS	LS	LS	+	extensive	red	2·5 c.c.	-
62	K.R. (a)	Δ.0.0.Δ.0.0.0.0.0	30	,,	420	·3	SS	LS	+		,,	dark red	-	0
63	H.H. (a)	Δ.0.0.Δ.Δ.Δ.Δ.0.0.0.0	31	,,	430	·3	MS	LS	+		,,	red	9·7 c.c.	0
64	B.P. (a)	Δ.Δ.Δ.Δ.0.0.0	27	,,	250	·3	MS	+			,,	dark red	1·0 c.c.	0
65	L.K. (b)	Δ.Δ.Δ.0.0	28	,,	450	·3	MS	LS	+		moderate	pink	6·2 c.c.	present
66	H.L. (b)	Δ.Δ.0.Δ.0.0.0	28	,,	450	·3	SS	+			extensive	,,	1·2 c.c.	present
67	B.T. (b)	Δ.Δ.0.0.Δ.0.0.0	33	,,	400	·3	LS	+			,,	dark red	-	0
68	N.M. (b)	Δ.Δ.0.0	57	,,	350	·3	SS	+			,,	,,	2·2 c.c.	present
69	H.M. (b)	Δ.0.Δ	?	,,	246	·3	MS	+			,,	,,	-	0
70	G.P. (a)	Δ.Δ.Δ.Δ.0.0.0.0.0	18	,,	430	·3	MS	LS	+		,,	,,	-	0

No.	Initials	Results of consecutive examinations	Days during which diphtheria bacilli remained in throat	Reaction in glucose broth	Animal inoculations						Results of Autopsy			Pseudo-diphtheria bacillus
					Wt. of guinea-pig in grammes	Dose of 48 hours broth culture in c.c.	Results. Days				Oedema	Supra-renals	Fluid in pleura	
							I	II	III	IV				
Catherine Street School														
71	L.S. (a)	Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.0.Δ.0.0.0.0	65	acid	250	·2	MS	+			extensive	red	-	0
72	D.G. (a)	Δ.Δ.0.Δ.Δ.Δ.Δ.Δ.Δ.Δ.0.0.Δ.0.0.0	33	,,	200	·3	SS	MS	+		,,	dark red	-	present
73	A.M. (a)	Δ.Δ.Δ.Δ.0.0.Δ.Δ.0.0.0	30	,,	250	·3	SS	+			moderate	,,	3·2 c.c.	0
74	E.B. (b)	Λ.0.0.0	13	,,	190	·3	N	N	N	14th day N	-	-	-	0
75	E.K. (b)	Δ.Δ.0.0.0	21	,,	245	·3	SS	+			extensive	dark red	3·2 c.c.	present
76	A.W. (b)	Δ.Δ.Δ.Δ.Δ.0.0.0.0.0.0	23	,,	200	·3	SS	+			moderate	,,	-	0
77	E.G. (b)	Δ.0.0	13	,,	160	·3	SS	+			extensive	,,	-	0
78	M.H. (b)	Δ.0.Δ.Δ.0.0.0.0	23	,,	225	·3	SS	MS	+		extensive haemorrhagic	red	-	present
79	A.S. (b)	Δ.Δ.Δ.0.Δ.0.0.0	23	,,	205	·3	SS	LS	+		extensive	,,	-	present
80	M.S. (b)	Δ.Δ.0.Δ.Δ.0.0.Δ.0.0.0	40	,,	160	·3	MS	+			moderate	dark red	-	present
81	N.S. (b)	Δ.Δ.0.Δ.0.0.0.0	19	,,	200	·3	SS	+			extensive	,,	1·2 c.c.	present
82	N.B. (b)	Λ.0.0.0	13	,,	205	2·0	N	N	N	14th day N	-	-	-	0
83	M.A. (b)	Λ.Λ.Λ.0.0.0	16	,,	200	·3	N	N	N	14th day N	-	-	-	present
84	W.C. (b)	Δ.0.0	47	,,	250	·3	SS	+			extensive	dark red	-	0
85	G.T. (b)	Δ.0.0.0	8	,,	200	·3	SS	SS	SS	12th day +	slight	pink	5·8 c.c.	0
86	F.E. (b)	Δ.0.0.0	16	,,	230	·3	MS	+			extensive haemorrhagic	,,	-	0
87	L.G. (a)	0.Δ.Δ.Δ.0.0.0	19	,,	200	·3	SS	+			,,	dark red	-	present
88	K.T. (a)	Δ.0.Δ.0.0.0	18	,,	150	·3	SS	MS	+		moderate	,,	-	present
89	G.T. (b)	Λ.0.0.0	20	,,	220	·3	SS	N	N	N	-	-	-	0
Abbey School														
90	M.A. (b)	Λ	?	,,	245	2·0	N	N	N	N	-	-	-	-
New Street School														
91	C.H. (b)	Λ.Λ.0.0.0	10	,,	200	2·0	N	N	N	N	-	-	-	0

St Barnabas School														
92	E.O. (a)	Δ.Δ.Δ.Δ	81	acid	200	·2	MS	+			moderate	dark red	trace	0
93	D.O. (a)	Δ.Δ.O.O	57	,,	260	·2	SS	+			,,	,,	-	present
94	G.O. (b)	Δ.O.O	?	not tested										present
Post Office														
95	A.O. (a)	Δ.Δ.Δ.Δ.Δ.Δ.O.O.Δ.O.O.O	16	acid	170	·3	MS	+			moderate	dark red	1·3 c.c.	0
96	J.H. (a)	Λ.Λ.O.Λ.Λ.Λ.O.Λ.O.O.Λ.O.O.O	15	,,	300	·2	N	N	N	14th day N	-	-	-	present
					290	2·0	N	N	N	N	-	-	-	
97	F.H. (b)	Λ.Λ.Λ.Λ.O.O.O.O	8	,,	260	2·0	SS	N	N	N	-	-	-	present
98	Y.B. (b)	Λ.Λ.Λ.Λ.O.O.O	9	,,	300	2·0	N	N	N	N	-	-	-	0
99	R.L. (b)	Λ.O.Λ.O.O.O	8	,,	200	2·0	N	N	N	N	-	-	-	present
Immediate contact unknown														
100	W.O. (a)	Δ.O.O.O	12	,,	210	·2	LS	+			extensive haemorrhagic	dark red	-	present
101	F.P. (a)	Δ. Fatal	-	,,	220	·2	MS	MS	LS	+	extensive	red	6·3 c.c.	-
102	M.S. (a)	Δ.Δ.O.O.Δ.Δ.Δ.O.O.O	35	,,	200	·2	LS	+			,,	dark red	1·5 c.c.	0
103	D.C. (a)	Δ. Fatal	-	,,	205	·2	MS	LS	+		,,	,,	-	-
104	O.O. (b)	Δ.Δ.Δ.Δ.Δ.O.O	70	,,	198	·3	MS	+			,,	,,	-	0
105	M.A. (a)	Δ.Δ.O.O.Δ.O.Δ.O.O.O.O	58	,,	245	·3	SS	+			haemorrhagic	,,	1·2 c.c.	0
106	G.W. (a)	Δ. Fatal	-	,,	245	·3	SS	+			extensive	,,	-	-
107	A.R. (a)	Λ.Λ.Λ.Λ.O.O.O	11	,,	210	·2	N	N	N	14th day N	-	-	-	present
					200	2·0	N	N	N	N	-	-	-	
108	M.R. (b)	Λ.Λ.O	20	,,	200	2·0	N	N	N	N	-	-	-	0
109	M.R. (b)	Λ	-	,,	not tested									
110	W.R. (b)	Λ.Λ.O	20	,,	205	2·0	N	N	N	N	-	-	-	present
111	D.R. (a)	O.Λ.Λ.Λ.Λ.O.O.Λ.O.O.O	15	,,	220	2·0	N	N	N	N	-	-	-	present
112	E.Y. (a)	O.O.Λ.O.Λ	?	,,	210	2·0	N	N	N	N	-	-	-	0
113	A.I. (a)	Λ.Λ.O.O	8	,,	230	2·0	N	N	N	N	-	-	-	
114	N.E. (a)	Λ.Λ.Λ.O	11	,,	300	2·0	N	N	N	N	-	-	-	0
115	J.C. (a)	Λ.O.O.O	1	,,	200	2·0	N	N	N	N	-	-	-	0

Most satisfactory results have been obtained by isolating the infected persons until the disappearance of morphologically typical diphtheria bacilli from the throat and nose, or other situations in which they may happen to be found. When the bacilli have persisted for a long period and show no signs of becoming reduced in numbers, pure cultures should be obtained and animal inoculations made. Should the bacilli turn out to be devoid of virulence, and difficulty met with in enforcing isolation, the patient may be discharged with the knowledge that non-virulent diphtheria bacilli have seldom, if ever, been known to give rise to clinical diphtheria or any other lesion, when transferred from one individual to another.

Many observers have taken one negative culture to be sufficient evidence for the disappearance of the bacilli and for the remission of quarantine, but the need for at least two or three consecutive negative cultures, before the patient can with reasonable certainty be declared to be free from the bacilli, has been clearly established.

A negative culture followed by one or more positive cultures, in which diphtheria bacilli are found, is of fairly frequent occurrence in examinations for release from isolation. The preceding table shows the results of the examinations of 112 infected persons during an outbreak at Cambridge (1904). The nature, the result of each examination in regard to the presence of the diphtheria bacillus and Hofmann's bacillus, the period of persistence, and the effect of inoculation experiments are given in each case.

Premature misleading negative cultures may be due to several causes.

In the first place less care is probably exercised in taking the specimens than in procuring swabs for diagnosis when the physician is most anxious about the case. Then the membrane, which previously formed a guide to the proper point, has vanished, and the swab may not be taken from that part of the mouth in which the bacilli are present, and the bacilli are generally fewer in number. At the particular moment when the culture is taken they may have disappeared temporarily from the surface of that part of the mucous membrane reached by the swab, only those remaining which are ensconced in folds or follicles or other inaccessible recesses, from which they may emerge later.

Wolff (1895) was the first to call attention to the presence of diphtheria bacilli in accessory sinuses of the nose. He examined these sinuses in 22 fatal cases of diphtheria, and found diphtheria bacilli in 12 cases. They were discovered once in the frontal sinus, six times in seven examinations in the sphenoidal sinus, and twelve times out of fifteen examinations of the antrum.

Councilman, Mallory, and Pearce (1901) found diphtheria bacilli in 21 (40 %) out of 52 cases of inflammation of the antrum, and in 19 (51 %) out of 38 examples of middle ear disease following diphtheria. These observations though made on fatal cases point to the possibility of the bacilli lurking in such situations, and subsequently finding their way thence into the pharynx.

Even though the bacilli are present in the throat they may not be removed on the swab; or even if they reach the swab in small numbers they may fail to be transferred on to the serum. They may even reach the serum and either fail to grow, or be overgrown by other forms. Failure to grow may be due to the presence of an antiseptic, if the swab has been taken too soon after its application to the throat, or, if the medium has not been very recently prepared, to the surface being too dry, etc.

Overgrowth of the colonies of the diphtheria bacillus may be due to the presence of numerous other organisms, or more frequently to the presence of rapidly growing film-forming bacilli.

Finally, although present on the culture, the bacilli may not be detected owing to the smallness of their numbers or insufficient examination (see p. 334).

Hill (1898) observed that 40 % of those officially released by two consecutive negative cultures yielded single negative cultures followed by positive cultures during their course, but states that at the Boston City Hospital not more than 3 % of diphtheria cases yielded positive cultures following two consecutive negative cultures.

Cobbett's (1901) records show that in 37 % of cases negative were followed by positive cultures. Rickards (1906, p. 27) has stated that 22 %, out of nearly 2000 persons released by the laboratory, showed a negative culture followed by a positive culture. Many similar observations dealing with smaller numbers are to be found in the literature.

During the last four years the writer has made cultivations from the throats and noses of 331 persons, convalescents and infected contacts, until the diphtheria bacilli have disappeared as evidenced by three consecutive negative examinations. Of these persons 127 (38·3 %) gave premature negative followed by positive cultures, many of them on more than one occasion. A single negative followed by the finding of diphtheria bacilli occurred 112 times, two consecutive negatives 66 times, and three consecutive negatives 17 times. In one instance four consecutive negatives followed by a positive culture were recorded.

The opportunity but rarely occurs of examining after a period of

several months persons who have been released from isolation after one or more negative bacteriological examinations. In a few instances the writer has been able to make such examinations and the results are given in the following table (p. 417).

The later cultures from 32 out of the 35 cases quoted showed no diphtheria bacilli. One of the children in whose throat the bacilli were found suffered from a second mild attack, for which no source of infection was traced. The other two were again discovered as healthy contacts in small outbreaks of the disease. In the first case diphtheria occurred amongst the members of the child's family, and in the second amongst the children attending the same school. In both cases it is probable that the child mentioned was the source of infection. The bacilli derived from both on the second occasion were fully virulent.

These records demonstrate that in about 40 % of cases one negative examination is misleading and that even two or three consecutive negatives cannot be entirely relied on as indicating the final disappearance of the diphtheria bacillus. Although it cannot, therefore, be claimed that danger is entirely removed by two, or even three consecutive negative examinations, it is certainly very much reduced.

However clearly the risk is realised of allowing convalescent patients and contacts infected with virulent diphtheria bacilli to mix with the normal population, the practical difficulties of enforcing prolonged isolation of any kind are often considerable. In the case of Infectious Diseases Hospitals, the wards of General Hospitals, Institutions and Schools, attended by the children of the well-to-do classes, little difficulty seems to have been encountered by most investigators.

The most serious opposition is likely to be encountered in dealing with isolated cases in adults and outbreaks amongst the scholars of the public elementary schools.

In the first case both the patient and the physician not unnaturally become impatient when the bacilli persist for an unusually long time. The former frequently fails to understand that he can still be a source of danger long after all symptoms of the disease have disappeared and when he appears to be in perfect health, and the latter chafes also under what he or his patients sometimes regard as an implied reflection on his skill, the delay in official release after he has declared the patient fully recovered appearing as a reversal of his decision. This idea is based on a misconception so obvious and simple that it would be unworthy of mention were it not so widespread. The procedure to be adopted in cases of this kind must of necessity depend on the circumstances in each

Number	Bacilli	Original period of persistence	Date of later examinations	Result of later examinations
Clinical Cases.				
1.	Virulent	18 days	5 months	Negative.
2.	,,	27 ,,	10 ,,	,,
			12 ,,	,,
3.	,,	31 ,,	10 ,,	,,
			12 ,,	,,
			17 ,,	,,
4.	,,	18 ,,	16 ,,	,,
5.	,,	8 ,,	18 ,,	,,
6.	Not tested	21 ,,	19 ,,	,,
7.	Virulent	24 ,,	19 ,,	,,
			20 ,,	,,
8.	,,	10 ,,	19 ,,	Bacilli present[1].
9.	,,	27 ,,	20 ,,	Negative.
10.	Not tested	17 ,,	21 ,,	,,
11.	Virulent	62 ,,	21 ,,	,,
Infected Contacts.				
12.	Virulent	25 ,,	2 ,,	,,
13.	Non-virulent	30 ,,	2 ,,	,,
14.	Virulent	47 ,,	4 ,,	Bacilli present[2].
			5 ,,	,,
			23 ,,	Negative.
15.	,,	40 ,,	5 ,,	,,
16.	,,	39 ,,	5 ,,	,,
17.	Non-virulent	9 ,,	6 ,,	,,
18.	,,	8 ,,	8 ,,	,,
19.	Virulent	9 ,,	7 ,,	Bacilli present[3].
			18 ,,	Negative.
			19 ,,	,,
			37 ,,	,,
20.	Not tested	12 ,,	10 ,,	,,
21.	Virulent	33 ,,	10 ,,	,,
			11 ,,	,,
			17 ,,	,,
22.	,,	46 ,,	10 ,,	,,
			11 ,,	,,
23.	,,	60 ,,	11 ,,	,,
			17 ,,	,,
24.	,,	?	11 ,,	,,
25.	Non-virulent	8 ,,	14 ,,	,,
26.	Not tested	21 ,,	14 ,,	,,
27.	,,	30 ,,	19 ,,	,,
			20 ,,	,,
28.	,,	9 ,,	19 ,,	,,
			25 ,,	,,
29.	Non-virulent	122 ,,	19 ,,	,,
30.	,,	52 ,,	19 ,,	,,
			25 ,,	,,
31.	Virulent	48 ,,	20 ,,	,,
			25 ,,	,,
			49 ,,	,,
32.	Non-virulent	?	20 ,,	,,
33.	Virulent	20 ,,	20 ,,	,,
			25 ,,	,,
34.	,,	41 ,,	22 ,,	,,
35.	,,	24 ,,	30 ,,	,,

[1] Suffered from a second mild attack of diphtheria. No known cause of infection.

[2] Bacilli were still present when the patient was released. Four months after he developed the disease three cases occurred in his family.

[3] An outbreak occurred in the school attended by this child. Virulent bacilli, which lingered 33 days, were found in his throat. The disease broke out amongst his immediate neighbours, and he appears to have been the source of infection.

instance. If the patient's employment brings him into close contact with school children, or persons of susceptible age, it is clearly necessary to use every means to convince him of the necessity for keeping away from his work, until diphtheria bacilli can no longer be found. If on the other hand his employment is such that the probability of his spreading the disease is slight, it may be a mistaken policy to insist too much on his continued isolation, for by this action general objections may be aroused to the procedure in cases in which isolation is more necessary.

In the case of outbreaks of diphtheria amongst the scholars of the public elementary schools considerable difficulties may be encountered in efficiently isolating, and more especially in keeping isolated, the infected contacts and mild cases until free from infection, unless some plan of action has been arranged. In this case again compulsion may be fatal to success. Nothing can be done unless the people back up the measures. On the discovery of such cases the measures which have been arranged should be explained to the parents, and an attempt made to induce them *to allow the infected children to be satisfactorily isolated, until proved to be free from bacilli by three consecutive negative bacteriological examinations.* When diphtheria is prevalent the failure now and again to isolate and keep isolated a person in whom diphtheria bacilli have been found is not of great importance.

It is clearly impossible to bacteriologically examine everybody who may have by some chance caught the bacillus. And since some such persons must inevitably remain at large, one more or less will not greatly signify.

Nevertheless it is worth while taking a considerable amount of trouble, if we can only isolate a good proportion of these infectious persons, or at any rate keep them from school. That it is possible even in considerable outbreaks to carry into effect the measures which have been advocated, namely the examination of most persons who are likely to have come into contact with cases of diphtheria or infected persons, and isolate those who are discovered to be harbouring diphtheria bacilli, until evidence has been obtained by three consecutive negative bacteriological examinations of the disappearance of these bacilli, has been proved by the experience of the Colchester and Cambridge outbreaks (p. 427). In these outbreaks the hearty cooperation of the medical practitioners, school authorities, and the majority of parents was obtained, when the principles of the measures which were being undertaken had been explained to them. Wesbrook (1905) in Minnesota has also carried into effect similar measures in a number of instances.

This applies only to times when diphtheria is prevalent: at other times when none but sporadic cases occur it is possible no doubt to examine every contact, and very desirable to isolate all infected persons until free from diphtheria bacilli.

(6) *Records relating to the persistence of diphtheria bacilli in the throat and nose, etc.*

Some statistics have already been quoted (p. 236) showing that diphtheria bacilli may persist, and retain their full virulence, in the throats and noses of convalescents long after the disappearance of all clinical symptoms. Far longer periods of persistence have, however, been recorded by observers, who have only taken into consideration the morphological appearances of the bacilli. The following table shows the longest periods of persistence recorded by a number of workers.

Table showing the longest periods during which diphtheria bacilli have been found by various observers to persist in the secretions after an attack of diphtheria.

Observer	No. of days	Observer	No. of days
Prip (1901)	669	Russell (1899)	137
Meyer (1898)	547	Graham-Smith (1904) ...	122
Le Gendre & Pochon (1895) ...	458	Wesbrook (1905)	109
Fibiger (1897)	276	Cobbett (1901)	108
Schäfer (1895)	230	Minnesota (1900)	97
Woodhead (1901)	210	Müller (1897)	75
Belfanti (1894)	210	Abel (1894)	65
Dowson (1893)	200	Washbourn & Hopwood (1895)	63
Massachusetts (1900) ...	185	Martha (1895)	63
Macgregor (1898)	183	Glucksmann (1897) ...	49
Park (1900)	183	Sevestre & Mery (1895) ...	49
Williams (1896)	157	Symes (1895)	49
Jensen (1897)	183	Gladin (1895)	45
Hewlett (1896)	154		

Although exceptional cases such as these come under the notice of nearly all observers, diphtheria bacilli as a rule can no longer be found after a few weeks, and in many cases even a few days after the disappearance of the local lesions. The mean period of their persistence varies to some extent according to the observations of different workers.

Woodhead (1901) during 1895–6 examined a large number of cases treated at the Metropolitan Asylums' Board Hospitals, and found that the mean period of persistence was 51 days. In his tables he differentiates

Table showing the persistence of morphologically typical diphtheria bacilli in the secretions[1].

Period in days of persistence of bacilli	Woodhead	Massachusetts Board of Health (1896—1905)	Westbrook (1900)	Park	Prip (1901)	Minnesota Board of Health	Glucksmann	Scheller (1905)	Tobieson	Total
1—5	—	66	—	304	—	—	3	—	10	1073 (11·8)
5—10	229	65	—	176	118	17	—	75	10	
11—15	—	400	17	100	—	23	26	264	1	1846 (19·8)
16—20	353	518	27	—	106	23	—	—	1	
21—25	—	271	—	12	—	57	29	119	—	1825 (19·8)
26—30	710	490	22	4	51	48	11	—	—	
31—35	—	304	10	—	—	—	9	62	1	1494 (16·3)
36—40	947	140	—	—	20	21	—	—	—	
41—45	—	47	—	—	—	—	4	35	—	1023 (11·2)
46—50	851	78	—	—	—	6	2	—	—	
51—55	—	8	—	—	—	—	—	26	—	653 (7·2)
56—60	526	39	—	2	41	11	—	—	—	
61—70	375	35	—	—	11	—	—	18	—	428 (4·7)
71—80	249	17	—	—	—	—	—	—	—	266 (2·9)
81—90	170	11	—	—	4	—	—	—	—	185 (2·0)
91—100	107	8	—	—	—	—	—	8	—	125 (1·4)
101—110	81	4	—	—	6	—	—	—	—	85 (·9)
111—120	69	1	—	—	2	—	—	—	—	72 (·8)
121—130	30	—	—	—	5	—	—	—	—	30 (·3)
131—140	16	2	—	—	—	—	—	—	—	18 (·2)
141—150	11	1	—	—	—	—	—	—	—	12 (·13)
151—160	5	—	—	—	2	—	—	—	—	5 (·05)
161—170	7	—	—	—	—	—	—	—	—	7 (·07)
171—180	2	—	—	—	—	—	—	—	—	2 (·02)
181—190	2	1	—	—	—	—	—	—	—	3 (·03)
191—200	2	—	—	—	—	—	—	—	—	2 (·02)
201—210	2	—	—	—	—	—	—	—	—	2 (·02)
Over 210	4744	2506	87[2]	598	3[3] 309	208	84	607	24	9080
Mean	51·2	28·0	29	6·6	24·7	26·8	24·8	20·9	8·4	38·4

between patients treated with and without antitoxin, but no appreciable difference was observed between them. No other recorded observations show such a long mean period of persistence, the longest being 29 days and the shortest 6·6 days.

The figures given by several authorities, most of whom regarded one negative examination as sufficient evidence of the disappearance of the

[1] Some of these observers took one negative examination as sufficient evidence for the disappearance of the bacilli and some two consecutive negative examinations.

[2] In 12 cases the bacilli disappeared after the 35th day of the disease.

[3] Over 8, 11, and 22 months. Two sets of observations by Prip are included. In one set the figures are given in months.

bacilli, are summarised in the preceding table, p. 420. These records very conclusively demonstrate the impossibility of choosing any time limit by which a patient might with any reasonable certainty be considered free from infection.

The records which are quoted above refer only to the microscopical examination of cultures taken from convalescent patients. They show that in three weeks about 30 % of patients are free from morphologically typical diphtheria bacilli. In 20 % the bacilli persist for four weeks, in 16 % for five weeks, and in 11 % for seven weeks. One per cent. harbour them for 15 weeks, and in exceptional cases they remain in the throat for thirty weeks, though even more prolonged periods of persistence are recorded.

It is of considerable interest to inquire whether any difference exists between the periods of persistence of virulent and non-virulent bacilli in clinical cases and contacts respectively. On these points the number of observations is very limited, and no marked differences are to be seen. The following table has been constructed from Cobbett's (1901) published observations and the writer's (published and unpublished), and includes only the records relating to cases in which three consecutive negative cultures were eventually obtained, and in which the bacilli were tested for virulence.

Period of persistence of bacilli in days	Notified cases		Infected healthy contacts	
	Bacilli virulent	Bacilli non-virulent	Bacilli virulent	Bacilli non-virulent
1—5	2	2	—	1
6—10	6	1	7	7
11—15	5	4	6	17
16—20	8	—	3	5
21—25	10	1	6	—
26—30	12	2	5	2
31—35	6	—	2	3
36—40	2	—	2	2
41—45	2	—	4	1
46—50	2	—	4	1
51—55	1	1	1	3
56—60	4	—	3	1
61—70	3	—	3	1
71—80	1	—	1	—
81—90	2	—	3	—
91—100	1	—	—	—
101—110	—	—	—	2
	67	11	50	46
Mean period	31·6 days	18·5	36·4	30·0

Certain persons were notified on very slight clinical grounds because diphtheria bacilli were found on bacteriological examination, but none of them suffered from typical diphtheria. The majority of the figures in the second column refer to such cases.

Persons harbouring virulent and non-virulent bacilli and clinical cases and contacts are separately considered.

According to the observations of Meikle (1906) the duration of the persistence of the bacilli is not appreciably affected by season, sex, age, number of days ill before admission, the amount and position of the membrane, the amount of antitoxin given, or the date of disappearance of the membrane.

The various statistics which have been quoted prove that diphtheria bacilli may persist for very long periods in the throats and noses of both convalescent patients and infected contacts, and retain their full virulence. They also show that in any given case it is impossible to foretell how long they will persist. Isolation for fixed periods of time can therefore be of little value, and gives rise to a false sense of security.

(7) *Attempts to hasten the disappearance of the bacilli.*

Since the discovery was first made that diphtheria bacilli remain in the throat after the clinical lesions have disappeared, efforts have been directed towards hastening their departure by the application of antiseptics. Great numbers of prescriptions have been recommended for use in various forms, as sprays, gargles, inhalations and as direct applications to the lesions. All these various methods and formulae have their advocates who consider that by their means the duration of the persistence of the bacilli is shortened, at least in many instances. During the last four years many of the methods which have been recommended have been given prolonged trials, but none have been found which give uniformly satisfactory results. In some instances good results seemed to follow, in others this was certainly not the case, and the writer has come to the conclusion that by antiseptic treatment the duration of the stay of the bacilli is not materially affected. Meikle (1906, p. 524) has recently made extensive observations on this subject, and gives the results in tabular form. He states that "no one antiseptic seems to be much better than another." Several other observers have also arrived at the same conclusion.

Some form of antiseptic treatment is however desirable for several reasons. In the first place the duration may perhaps be shortened

in some cases, and in the second the patients and their friends are better satisfied if some attempt is apparently being made to shorten the period of isolation. Thirdly, the application of antiseptics probably hinders the multiplication of the bacilli on the mucous surfaces, and also diminishes their vitality outside the body. Consequently the treatment, although it is unable to affect the bacilli, which are present in the crypts of the tonsils and other out-of-the-way situations, and therefore does not reduce the period of infectivity of the patient, is of benefit in limiting the power of infecting others, by its action on the superficial bacilli.

When swabs are taken from patients undergoing such treatment an interval of several hours ought to elapse between the application of the antiseptic and the taking of the swab, in order to allow the bacilli which are lurking in the crypts, etc., to regain the surface, and to prevent any recently applied antiseptic producing an inhibitory action on the culture.

It has occasionally been noticed that after an inflammatory lesion due to some other agent than the diphtheria bacillus these organisms disappear from the throats of healthy infected persons. In the case of a man, in whom the bacilli persisted in a few large crypts for an unusually long time after an attack of diphtheria, an attempt was made to hasten their departure by the application of irritants to these crypts. By means of small bent swabs small quantities of mustard oil were applied on several occasions to each of the enlarged crypts. This procedure resulted in an acute inflammatory process in the superficial portions of the crypts, but the bacilli nevertheless persisted in the deeper portions.

Wassermann (1902) has tried the experiment of giving tabloids made from the serum of horses immunised against several strains of living diphtheria bacilli. He claims to have been successful in hastening the disappearance of the bacilli by this method.

L. Martin (1903), by injecting the bacillary bodies intravenously or intraperitoneally into horses, obtained sera with marked agglutinating properties. He claims that this serum has, when applied locally, the property of causing a rapid decrease in the number of living bacilli in the throat. The best results are obtained by incorporating the dried serum with gum, and using the remedy in the form of pastilles.

In many instances the removal of enlarged tonsils, adenoids or nasal growths, which provide the bacilli with situations where they may multiply unharmed by antiseptic applications, might be of great benefit

in hastening their disappearance. Pegler (1905) stated that "he had several times found that the cause of this lingering presence of bacilli could be removed by carefully extirpating by means of morcellement every trace of unhealthy tonsil tissue in which crypts could be discovered." In other cases he had found adenoid, or naso-pharyngeal tonsillar tissue to be the offending cause of the trouble.

(8) *The results of the isolation of convalescents, mild cases, and infected contacts until the disappearance of diphtheria bacilli.*

(*a*) *Families.*

Park (1894) investigated the throats of the members of 14 infected families in which there were 48 children. In 50 % of these virulent diphtheria bacilli were found; 40 % developed later, to a greater or less extent, the lesions of diphtheria. It is noted in Park's paper that, in these families, the conditions were the best possible for the transmission of the bacilli from one to the other. In families where the case of diphtheria was well isolated, the bacilli were found in less than 10 % of the children.

(*b*) *Schools.*

One of the first attempts to stamp out diphtheria by the isolation of infected contacts was brilliantly successful. The credit is due to Fibiger (1897), who dealt with an outbreak in a gymnasium at Herlrifsholm in the Island of Zealand. In December 1894 there were three cases of diphtheria. Then came the Christmas holidays, during which the rooms, bed clothes, etc., of the patients were twice disinfected before the school reassembled. The scholars returned on January 7th, and on the 13th, a case of diphtheria occurred, quickly followed by five others. A bacteriological examination of all the healthy persons (134) was undertaken, with the result that diphtheria-like bacilli were found in 22. Further investigations were made to differentiate between the true and pseudo-diphtheria bacilli. It was then found that those (10) who harboured the true diphtheria bacilli were children who shared the same rooms as the diphtheria patients, and as a rule were the next neighbours. One of these children had suffered from the disease in December, but had recovered. One case occurred in a class which had not been affected, and it turned out that the brother who belonged to another class had carried the infection.

After the isolation of the diphtheria bacilli-carrying individuals no cases occurred for 1½ years. Some of these infected contacts retained the bacilli for long periods ; in one case virulent bacilli being found for nine months.

Gabritschewsky (1901) on two occasions checked outbreaks of diphtheria in large schools by the examination of the scholars, and the isolation of all in whom diphtheria bacilli were discovered.

Burnett's (1900) case, which has already been quoted (p. 311), affords an excellent example of diphtheria reintroduced into a school by a convalescent patient. In this case the patient was thought to be free of diphtheria bacilli, but they were again found after his return to school. Peck (1901) also gives an instance illustrating the reintroduction of the disease by a contact. In a school with 50 boarders and 50 day scholars two boarders were found to have sore throats on October 5th. Swabs were taken and diphtheria bacilli discovered. The day scholars were immediately dispersed, and one boarder (A. B.) was allowed to go home on the understanding that she was not to return till bacteriologically free. A third case occurred on October 8th. All the persons in the house, including the boarders, teachers, and servants were then examined, and all but two were found to be free from diphtheria bacilli. The three cases and two infected contacts were isolated.

On November 5th all the scholars reassembled and all were bacteriologically free. A. B. returned on November 12th but went home on November 16th, and on November 19th developed diphtheria. It was then ascertained that she had not been examined, but had been using an antiseptic spray. On November 18th five boarders were suffering from slight ailments and three were found to have diphtheria bacilli ; swabs from the rest of the school showed 28 children harbouring the bacilli.

(c) *Institutions.*

Berry (1900) gives a very interesting account of an outbreak of diphtheria at the London Orphan Asylum in 1898, which was arrested by the examination of the children and the isolation of those infected. At that time there were 350 boys and 200 girls in the Asylum, who were kept apart, and except at the Summer and Christmas holidays had little communication with the outside world. A case of true diphtheria occurred amongst the girls shortly after the Christmas holidays (Feb. 25th). A series of mild sore throats followed till

March 27th, when another case of clinical diphtheria occurred. At the same time three children with sore throats were found to be harbouring diphtheria bacilli, and were isolated. Nevertheless numerous cases of sore throat followed until April 30th, when five children developed diphtheria. At this time there were 76 cases of sore throat. Washbourn then examined all children (142) showing any abnormality of the throat, and discovered diphtheria bacilli in 19 of them. These children were isolated and not allowed to mix with the healthy girls until free from diphtheria bacilli. From that time no further cases of diphtheria or sore throat occurred.

Wesbrook (1905) records the successful treatment of an outbreak amongst the inmates of the State School for the Deaf at Faribault in which unusual difficulties had to be overcome. Twenty clinical cases were under treatment amongst the 280 inmates when the measures were put into force. All persons connected with the institution were examined and all infected individuals, who included 28% of the children, isolated. All who showed suspicious bacilli (other types than D, C, or A) were re-examined, and if they failed to show D, C, or A on the second examination were released.

The infected individuals were not allowed to mix with the others until three successive negative throat and nose cultures had been recorded. Only two new clinical cases occurred after the measures were put into force and the disease was effectually eliminated, although there were many difficulties incidental to overcrowding, some difficulties in instructing the pupils, and some due to the facts that the pathological conditions of the nose and throat which originally gave rise to the deafness were favourable to diphtheria infection, and that the use of the sign language employed increased opportunities for infection from the hands.

(*d*) *Hospital wards.* (See also Chapter XII.)

Garratt and Washbourn (1899) from their observations at the London Fever Hospital from March 1896 to December 1898 come to the conclusion "that post-scarlatinal diphtheria is due to the introduction of unrecognised cases of diphtheria into scarlet fever wards, and that this can only be obviated by systematic bacteriological examinations of all cases on admission and by the separation of those in whose throats diphtheria bacilli are found." They considered that Hofmann's bacillus had no connection with diphtheria, and did not separate persons showing this organism.

These observers succeeded in producing a great reduction of post-scarlatinal diphtheria by isolating those cases in which diphtheria bacilli were found (see p. 439).

Soerensen (1898) had in the previous year called attention to the rapidity with which diphtheria bacilli spread amongst scarlet fever patients, when a case of diphtheria or persons infected with diphtheria bacilli are introduced into a ward.

Hutchens (1906) records an outbreak of diphtheria in a scarlet-fever ward. On examining the throats of all the inmates diphtheria bacilli were found in 23% and Hofmann's bacilli alone in about one-half the remainder. "The former were all isolated, and no further cases occurred."

The instance given by Cuno (1902) of diphtheria spread in wards by a sister harbouring virulent diphtheria bacilli has already been quoted (p. 316). On the discovery of this fact and the isolation of the sister the outbreak came to an end.

Peters (5. VIII. 1907) completely checked an outbreak amongst the members of the staff of the Nottingham General Hospital by the isolation of infected contacts.

(*e*) *Towns.*

Two remarkable instances of the effects of efficient examination and quarantine in dealing with considerable outbreaks are given in the Report of the Massachusetts State Board of Health (1900). At Waltham on the opening of the schools an epidemic began which spread to all sections of the city. In November there were at one time 50 cases, and several new cases were being reported daily. The throat of each school child in the city was examined and cultures taken from all suspicious cases. By this means 22 children were found to be suffering from mild diphtheria. "These children were isolated and kept in quarantine until cultures showed them to be free from infection. The effect of this procedure upon the spread of the epidemic was soon apparent, and before the Christmas vacation the number of new cases was comparatively small."

At West Springfield during the last five months of 1899, 29, 44, 37, 25 and 24 cases had been notified, and 18 cases occurred in January 1900. Quarantine, until cultures showed the absence of diphtheria bacilli, was advised by the State Board of Health at the beginning of February. Only two cases occurred in February and none in March.

Cobbett (1901) in 1900 dealt with a serious outbreak of diphtheria at Cambridge by the examination of contacts and the isolation at an Isolation Home of almost all those found to be infected. Prophy-

lactic injections of antitoxin were also given to a considerable number of the latter. The outbreak soon subsided after these measures had been put into operation. He emphasises the fact that diphtheria bacilli were only found in actual cases of diphtheria, or among those who had come directly into contact with such cases. A recurrence of the disease in the spring of the following year was also similarly treated.

Cobbett (23 XI. 1901) considers "that the duty of discovering, isolating and disinfecting the former class of persons (infected contacts) is becoming more and more the urgent duty of the sanitary authority. For the fact that they are not scattered broadcast throughout the community as was once supposed, but are confined to the class of persons conveniently called 'contacts,' renders their discovery a practical possibility and offers a fair prospect that at least the great majority of them may in the near future be subjected to isolation and antiseptic treatment with immense advantage to the public health."

Exactly similar measures were employed in combating a widespread outbreak of diphtheria at Colchester in 1901 by Graham-Smith (1902), but in this instance a very large number of cases had occurred before the measures were put into force.

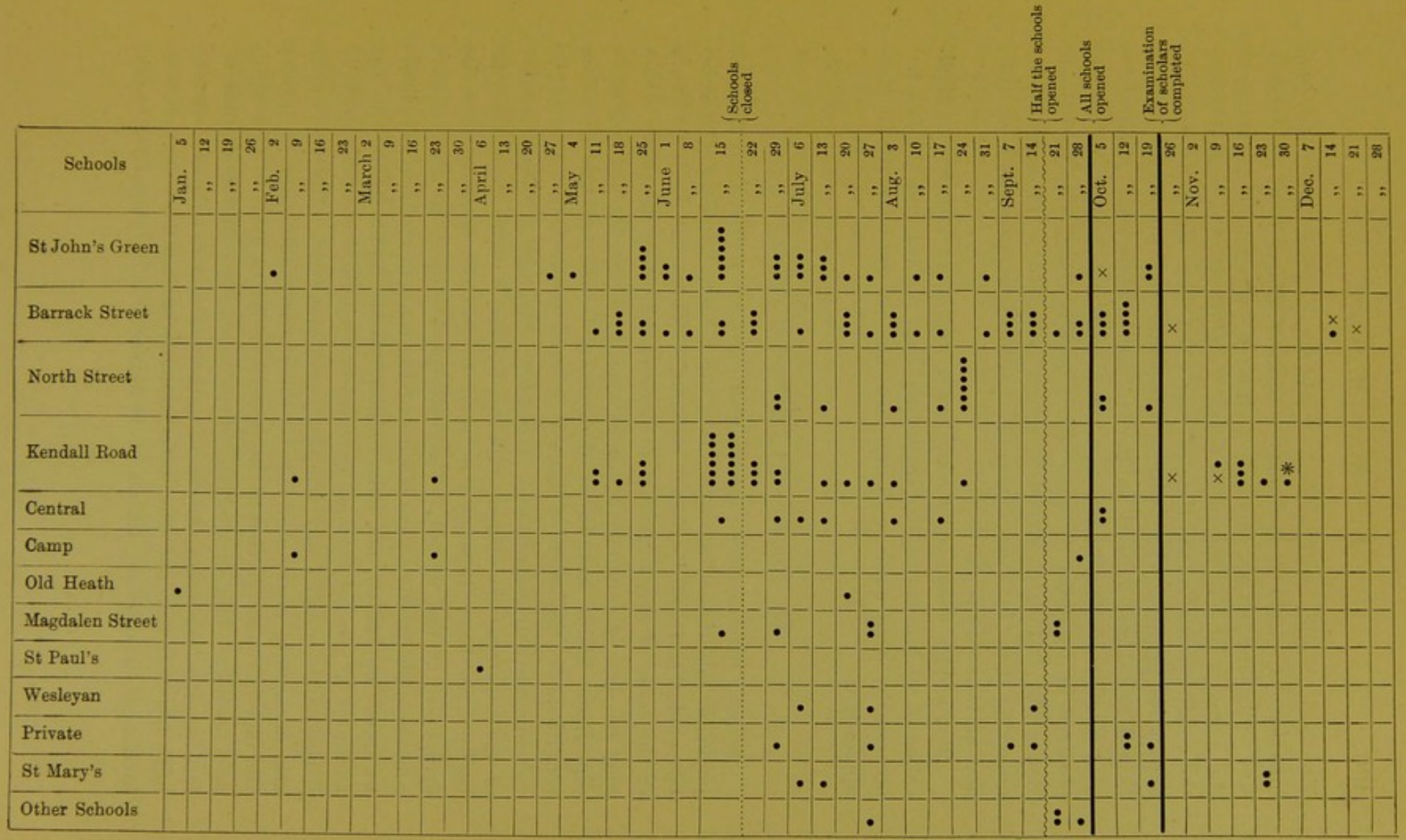
The accompanying chart (p. 429) illustrates better than any description the success of the system. As may be seen in spite of the closure of the schools on June 15th, six cases a week on the average continued to be notified for 18 weeks up to October 19th. By this date all the probable contacts had been examined and the infected children quarantined. The result was that, in spite of the re-opening of the schools at a time of year when diphtheria might be expected to increase, the disease almost completely disappeared. A small outbreak occurred at the Kendall Road School, which was immediately stamped out by the re-examination of this school. Three other cases of true diphtheria also were notified during the remaining 10 weeks of the year.

In 1903 Graham-Smith (1904) again employed the same measures in an outbreak at Cambridge. In this case the disease occurred amongst the scholars of certain schools (3) and amongst the inmates of certain institutions (3). Each of these outbreaks was successfully dealt with in turn[1].

Wesbrook (1905) reports several widespread epidemics most successfully combated by following up and examining all cases, and all rumours and suspicions of possible cases. He also examined all school children, and excluded from school each child and each teacher who showed

[1] See Tables, pp. 408—413.

Chart showing the results of the isolation of mild unrecognised cases and infected contacts amongst the School Children of Colchester during an outbreak in 1901.



• indicates a notified case of diphtheria.

× indicates a notified case of diphtheria in which no diphtheria bacilli were found.

⁂ re-examination of Kendall Road School completed.

diphtheria bacilli, until he and all his family were found free from infection. In 1900 Park Rapids, a town of almost 1000 inhabitants, was badly infected, not only school children, but adults being attacked. As a result of the measures taken "diphtheria disappeared under the conditions and at the time of year when there was every reason to expect its increase. The willing co-operation of the Town Council, School Board and general public was easily secured and retained."

"Similar methods have been employed and comparable results obtained in a number of other places, notably Grand Rapids, Benson, Bowerville, Duluth, Robbinsdale, Heron Lake, etc." He concludes by observing that "in Minnesota public sentiment supports such methods and at times demands them."

The sudden cessation of considerable outbreaks without the application of such measures has frequently been noticed, and the fact has occasionally been used as an argument against the necessity for their application.

The Prophylactic Injection of Antitoxin.

Several investigators have claimed excellent results from the prophylactic injection of antitoxin into the healthy members of infected households and into "infected contacts." There can be little doubt of the value of this treatment of persons exhibiting symptoms, which may be due to commencing diphtheria, in whose throats and noses diphtheria bacilli have been demonstrated. Except under certain conditions the injection of healthy infected contacts can scarcely be recommended in considerable outbreaks. Although a certain number of these persons do develop clinical diphtheria some days after the bacilli have been discovered in their throats, the majority show no symptoms of the disease. The attempt to inject all the infected contacts adds greatly to the labours of the workers, and is always liable to arouse opposition, not only to prophylactic injections, but to the measures in general amongst ignorant or prejudiced parents, especially if the children suffer any temporary inconvenience. The expense is also necessarily heavy and falls on the sanitary authority. A further and more serious objection to the practice is that it leads to a feeling of false security. The injected individuals do not often contract diphtheria, but nevertheless retain the bacilli in their throats as long as those who have not been injected, and unless carefully isolated are liable to act as carriers of the disease. Instances are also recorded (Jump, 1902, etc.) of

persons who have received prophylactic injections developing the disease weeks after infection without reinfection. Peters (1907) found that seven out of 21 infected contacts, who received prophylactic doses of antitoxin (500 units), developed diphtheria within three weeks. If all the infected contacts that are known are under supervision, and instructions have been given to the parents or guardians immediately to notify any ailments amongst them to the medical authorities, prophylactic injections are unnecessary, since antitoxin can be at once given on the occurrence of suspicious symptoms. On the other hand prophylactic injections of antitoxin ought to be given in cases where medical attendance cannot easily be procured and the parents are likely to await the development of a serious condition before sending for the medical man.

Summary of Chapter XI.

The same general preventive measures are employed in dealing with outbreaks of diphtheria as in combating other infectious diseases. The measures include the notification of all persons suffering from typical and atypical forms of the disease, and their efficient isolation, and the disinfection of all articles used by them. Most authorities consider that healthy persons, found to be harbouring diphtheria bacilli, should not be notified but treated separately. During outbreaks the public elementary schools are generally closed, but this ought to be preceded by the bacteriological examination of the children. Special preventive measures based on bacteriological findings are now frequently employed, especially in the case of schools, hospital wards and institutions, and have also been successfully used in dealing with larger outbreaks in towns. These measures include the bacteriological examination of all suspected cases, and of those who have come into contact with them, and the isolation, or at least exclusion from intercourse with the susceptible members of the community, of all found to be harbouring diphtheria bacilli. Isolation should be enforced, whenever possible, until the bacilli have disappeared as evidenced by at least two, and if possible three, consecutive negative bacteriological examinations. Occasionally the bacilli persist for very long periods of time, particularly in persons with large tonsils, etc. None of the numerous antiseptic applications, which have been employed, have proved very successful in hastening their disappearance. Except when combined with efficient bacteriological examinations indiscriminate prophylactic injections of antitoxin cannot be recommended.

CHAPTER XII.

POST-SCARLATINAL DIPHTHERIA.

The prevalence of post-scarlatinal diphtheria. Origin. Treatment in the same hospitals of scarlet fever and diphtheria. Introduction of unrecognised cases. Results of bacteriological examinations. Spread of diphtheria in scarlet-fever wards. Prevention.

The Prevalence of Post-scarlatinal Diphtheria.

THE liability of scarlet fever patients to develop diphtheria has long been known, and the literature relating to the connection between the two diseases is very extensive. Although patients suffering from diphtheria may subsequently develop scarlet fever, or both diseases appear simultaneously in one subject, in the great majority of instances in which these two diseases occur in the same patient diphtheria follows the attack of scarlet fever.

Numerous recent English and foreign statistics show that scarlet fever patients treated in hospitals are liable to develop diphtheria in greater or less numbers, and their liability to this complication is universally recognised. Consequently it seems unnecessary to give at length the figures relating to this subject.

For several years returns have been made by the hospitals of the Metropolitan Asylums Board on the subject of this complication. Previous to 1895 only cases of scarlet fever which showed clinical diphtheria, having membrane on the fauces or exhibiting laryngeal symptoms, were designated post-scarlatinal diphtheria; since that year all cases of secondary throat illness associated with the diphtheria bacillus have been returned as diphtherial, including those which would from the clinical appearance alone have been regarded as simple tonsilitis.

In the year 1895 there consequently appears a sudden and large increase in the incidence of secondary diphtheria among the patients in the scarlet fever wards of these hospitals.

	1891	1892	1893	1894	1895	1896	1897	1898	1899	1900
Scarlet fever cases completed	5,444	11,326	14,867	12,637	10,422	15,054	15,250	12,771	13,327	10,749
Cases of post-scarlatinal diphtheria	99	217	207	210	453	705	796	661	692	405
Percentage incidence	1·8	1·9	1·3	1·6	4·3	4·6	5·2	5·1	5·1	3·7

Pugh (1902) worked out the seasonal incidence of these cases for the five years 1896–1900. He states that "if the number of cases of post-scarlatinal diphtheria, developing in each month in the five years under consideration, be averaged and corrected for the mean daily number of scarlet fever patients under treatment in each of these months, it will be found that the incidence of this complication does not follow the seasonal variation of diphtheria in the Metropolis, nor does it appear to depend on whether the hospitals are full or the reverse."

"In calculating the incidence of post-scarlatinal diphtheria on the the number of patients discharged and dead an important correction is necessary. Returns made in the years 1899 and 1900 show that the patients treated to recovery or death in the Board's town institutions (acute hospitals) have been in hospital about 68 days on the average, while patients who have completed their recovery or died at the convalescent hospitals have been, on the average, 31 days at the town hospital and about 48 days at the convalescent institution. If the calculation be made on 'patient days' (on the 'foot-pound' principle), it will be found that the liability to post-scarlatinal diphtheria at the convalescent hospitals is about two and one-third times as great as at the town hospitals."

The origin of post-scarlatinal diphtheria.

Pugh (1902) recently discussed the origin and mode of dissemination of post-scarlatinal diphtheria in the Metropolitan Asylums Board Hospitals, basing his conclusions on own observations, the statistics of the Board, and the previous literature. This section, in which the principal reasons are considered which have been suggested for the occurrence of secondary diphtheria in scarlet fever wards, is mainly based on this author's paper.

(1) *Sanitary defects.*

"As advances have been made in our knowledge of the bacterial origin of diphtheria, the belief, once generally held, that defective drainage played an important part in disseminating this disease has gradually waned. However, it may be as well to recall that Sweeting

in 1893, investigated this point in connection with the Board's hospitals, and found that post-scarlatinal diphtheria had prevailed in like degree in hospitals with ventilated and in those with unventilated soil-pipes; in hospitals with automatic flushing apparatus, and in hospitals without such appliances; in hospitals with elaborate systems of ventilation and disconnection and in hospitals where these were of the most meagre and incomplete kind. In fact, the diversity was so great that no common factor of drainage defect could be pointed to as explaining the long continued yearly recurrence of this condition of post-scarlatinal diphtheria."

(2) *The treatment in the same hospital of the two diseases.*

"It is but natural that a layman, unacquainted with the administration of a fever hospital, should, when he hears that his child, convalescent from scarlet fever, has developed diphtheria, forthwith concludes that infection has been derived from cases of diphtheria treated in the same hospital." This opinion has to some extent been shared by members of the medical profession.

Now if, as supporters of this theory have held, the treating in the same hospital of the two diseases is the main cause of post-scarlatinal diphtheria, one would expect it to be of comparatively rare occurrence in hospitals reserved entirely for the treatment of scarlet fever. That this is not so is evident from the experience of the North Eastern and Gore Farm Hospitals. The former during the five years 1896–1900 received only patients certified to be suffering from scarlet fever, but 160 cases of secondary diphtheria occurred, while at the latter, which during 1897–8 received scarlet fever convalescents only, 273 cases of diphtheria were recorded.

This point is further emphasised by a comparison of the incidence of post-scarlatinal diphtheria at the Northern Hospital and Gore Farm during the years 1896–8. At the former convalescents from both diseases were admitted and at the latter only scarlet fever convalescents, yet the incidence of post-scarlatinal diphtheria in the two institutions was almost identical, namely 4·9 % at the former and 4·5 % at the latter.

Pugh (1902, p. 292) remarks that "it may, therefore, be regarded as proved, so far as statistics are able to help one, that the aggregation upon the same site of the two diseases is not an important factor in the etiology of post-scarlatinal diphtheria. Indeed, since diphtheria spreads solely through intimate contact with the source of infection, it can extend to the scarlet fever wards only in consequence of imperfect

separation of the convalescents or through conveyance there by members of the staff."

The first of these two means of infection, if it occurs, can easily be remedied, and owing to the methods usually adopted can scarcely be of much importance. Practically the only persons involved are therefore the medical officers and the nurses. The former, however, are not brought into sufficiently close contact with their patients to encourage the belief that they serve as sources of infection with any degree of frequency. The nurses, on the other hand, owing to the more intimate relations which exist between them and the children, are more important factors in the spread of the disease.

Many instances are recorded in the literature on diphtheria of the infection of doctors and nurses with diphtheria bacilli, and instances have already been quoted (p. 316) of the spread of the disease by this means.

Pugh (p. 292) cites an interesting example: "Prior to the opening of our diphtheria wards there was an isolation building in this hospital, used for cases erroneously diagnosed as scarlet fever, containing four separate rooms, which were looked after by a single nurse. In one was a child with bronchitis; in another a patient suffering from diphtheria. The latter died on November 18th, five days after admission. On December 3rd, the bronchitic child, who had not yet left his bed, developed laryngeal diphtheria necessitating tracheotomy. No source of infection appeared possible save by the medical or nursing staff. Cultures were made from the throats of all who had been in contact with the child, and from one nurse, who had been in attendance on the diphtheria case of a fortnight before, virulent diphtheria bacilli were obtained. She had throughout had no sore throat, and the tonsils showed only chronic enlargement."

(3) *The introduction of unrecognised diphtheria.*

According to most authorities the introduction of mild and unrecognised cases of diphtheria into scarlet fever wards is the cause of post-scarlatinal diphtheria. "Among the cases received into fever hospitals certified as scarlet fever, a few can be readily recognised clinically as uncomplicated diphtheria. A larger class is that in which there is on admission evidence only of tonsilitis, for patients have not infrequently lost by the time they arrive at the hospital the other signs upon which the practitioner founded his diagnosis, and yet many of these are proved subsequently, by the occurrence of desquamation, to be

suffering from the disease certified. Owing to the limited number of isolation rooms, a considerable proportion of these cases of apparent tonsilitis are admitted for observation into the scarlet fever wards, and one of mild diphtheria might thus be the origin of an outbreak of post-scarlatinal diphtheria.

Another class of case is that of double infection. Occasionally in addition to the signs of scarlet fever, the patient presents undoubted diphtheritic membrane in the throat. These cases, however, must not be confounded with a much larger number in whom a condition of throat more or less simulating diphtheria is found....Cases of the combined diseases may occasionally be admitted in which the local evidence of diphtheria is so slight, or else so masked by the lesions of scarlet fever, as to escape recognition. Finally, as is well known, there may, under certain circumstances, be present in the throats which are apparently quite healthy virulent bacilli, which are capable of causing diphtheria in other patients."

The results of bacteriological examinations of scarlet fever patients on admission into hospital.

(1) *Throat.*

Pugh (p. 295) examined bacteriologically the throats of 420 unselected cases admitted into the North Eastern Hospital, certified as scarlet fever, of which two proved to be uncomplicated faucial diphtheria. From the throat of a patient suffering from scarlet fever and considerable obstruction to respiration, and from the throats of two others suffering from fibrinous rhinitis, virulent diphtheria bacilli were obtained. From 17 (4·5 %) of the remaining 415 cases diphtheria bacilli were cultivated, and five of these strains were tested for virulence with negative results, although they all stained with Gram's and Neisser's solutions and rendered neutral litmus glucose broth acid.

Garratt and Washbourn (1899) examined the throats of 666 cases of scarlet fever patients admitted under their care at the London Fever Hospital from March 1896 to December 1898, and found bacilli morphologically resembling *B. diphtheriae* in eight (1·2 %). Goodall (1896) found long diphtheria bacilli in the throats of six (7 %) out of 87 scarlet fever patients.

(2) *Nose.*

Pugh (p. 303) examined by cultures the noses as well as the throats of the 420 scarlet fever patients previously mentioned.

Diphtheria bacilli were obtained from the nose of one of the two diphtheria patients, and from the noses of the two cases of fibrinous rhinitis. Virulent diphtheria bacilli were also obtained from the nose, but not from the throat, of a child whose two sisters were suffering from diphtheria.

The remaining 414 cases presented on careful inspection no evidence of either faucial diphtheria or fibrinous rhinitis; nevertheless from the nasal cavities of 33 morphologically typical diphtheria bacilli were obtained. In 10 of these cases the organisms were present also in the throat, but in 23 they were present in the nose alone. Three of these strains were tested on animals and all were found to be non-pathogenic.

These observations show that diphtheria bacilli are frequently present both in the throats and noses of scarlet fever patients admitted into the London fever hospitals. In the case of those who show clinical evidences of faucial diphtheria or are suffering from fibrinous rhinitis the bacilli are generally virulent, but many of the bacilli obtained from patients without diphtheritic lesions are non-pathogenic to animals, and are probably incapable of giving rise to diphtheria.

The spread of diphtheria in scarlet fever wards.

The observations of Müller (1896) and others have shown that diphtheria bacilli rapidly become disseminated amongst patients in scarlet fever wards and amongst convalescents from this disease, when a patient suffering from diphtheria is introduced amongst them.

Chronic rhinitis with sore nostrils, a varying amount of discharge, and a tendency to the formation of pustules on parts of the body, is a fairly common sequel of scarlet fever. Todd (1898) called attention to the fact that this form of "external rhinitis" is sometimes due to the diphtheria bacillus (see p. 360).

Pugh (p. 305) says that "in similar cases at the North Eastern Hospital it is not uncommon to find that the condition is, in reality, fibrinous rhinitis," and gives an interesting account of an outbreak. "In a convalescent scarlet fever ward, occupied chiefly by girls of five, a child who developed rhinorrhoea was found to have membranes on the

septum and the roof of the bony orifice of each nasal cavity; on the left tonsil was a small area of deposit. Cultures showed the presence of diphtheria bacilli in the throat and nose. The bacilli from one nostril were found to produce a strongly acid reaction in sugar broth, and 2 c.c. of a 48 hour broth culture proved lethal to a guinea-pig at the end of the second day, the result being prevented in another guinea-pig by the simultaneous injection of antitoxin. In three rounds of cultures made from the other 25 patients, who were kept in bed meanwhile, no fewer than 10 were found to have acquired the diphtheria bacillus. The spread of infection appeared to have been assisted by the fact that among the toys of the ward were school slates which were used indiscriminately by all. Well marked fibrinous rhinitis was present in two; their throats were normal, although, in one, diphtheria bacilli were present there also. The other eight children had diphtheria bacilli in the throat, which presented no abnormality except in one case, where there was a thin sheet of membrane on the right tonsil. In two the organisms were present in the nasal cavities also; these clinically appeared normal. Bacilli from each of the cases of fibrinous rhinitis, and from one of the healthy throats, were found to render glucose broth acid, and 48 hour broth cultures in 2 c.c. doses proved fatal to guinea-pigs in 48 hours, while in each case this effect was prevented in control animals by antitoxin."

Williams (1901) also found diphtheria bacilli in about 22 % of cases of post-scarlatinal rhinorrhoea. Of the 12 typical diphtheria bacilli tested for virulence four were found to be virulent and eight non-virulent.

The prevention of post-scarlatinal diphtheria.

This subject divides itself naturally into two parts, (A) the prevention of the introduction of virulent diphtheria bacilli into a ward, and (B) the prevention of the spread of the organisms amongst the patients.

A. *The prevention of the introduction of diphtheria bacilli.*

(1) *By members of the staff.*

"It follows from the observations which have been recorded that the transference of nurses from the diphtheria to the scarlet fever side of a hospital should not occur more frequently than can be helped, and

that those who have been working in wards containing diphtheria or post-scarlatinal diphtheria patients should not be put on duty in scarlet fever wards unless they have been proved by culturing to be free from the means of infecting their charges with diphtheria."

A likely means both of acquiring and of distributing infection would seem to be the fondling and kissing of children; the rule, understood, in every hospital, that no child should be kissed by a nurse is without doubt very frequently broken.

(2) *By patients.*

A careful inspection of the throat on admission, with bacteriological examination in cases of doubt, is customary at all fever hospitals. That a similar examination of the nasal cavities is equally important is evident from some of the cases which have been quoted.

The important question arises as to whether a routine bacteriological examination of all patients should be made on admission. Such an examination of the throats was advocated by Garratt and Washbourn (1899) as a method of preventing post-scarlatinal diphtheria. During a period of 27 months from March 1896 to December 1898 they examined bacteriologically the throats of all the scarlet fever patients admitted under their care at the London Fever Hospital, and isolated all those in whom diphtheria bacilli were found, and by this means obtained a great reduction of the number of cases of post-scarlatinal diphtheria. The following table gives the proportion of such cases at the London Fever Hospital and in the Hospitals of the Metropolitan Asylums Board from 1893 to 1897.

	London Fever Hospital			Hospitals of the Metropolitan Asylums Board		
	Scarlet fever admissions	Diphtheria admissions	Post-scarlatinal diphtheria	Scarlet fever admissions	Diphtheria admissions	Post-scarlatinal diphtheria
1893	764	5	·52 %	14,548	2,848	1·40 %
1894	294	25	·34 %	11,598	3,666	1·90 %
1895	516	52	2·71 %	11,271	3,635	3·62 %
1896	637	65	·47 %	15,172	4,508	4·62 %
1897	431	45	·23 %	15,241	5,673	5·22 %
1898	325	40	0 %			

In consideration of the excellent results obtained by these observers it would seem advisable to examine the throats and noses of all cases on admission at times when diphtheria is prevalent, and to place all those found to be infected in separate wards. By this means the probability of the introduction of diphtheria amongst the mass of scarlet fever patients would be greatly lessened.

B. *The prevention of the spread of diphtheria bacilli amongst scarlet fever patients.*

When the virulent diphtheria bacillus has invaded a scarlet fever ward, as evidenced by the occurrence of a case of secondary diphtheria, the same measures as are applied in outbreaks of the disease in schools, etc., are indicated. "All the patients should be kept in bed, and inter-infection by their toys, handkerchiefs, etc., prevented, while the condition of their throats and nasal cavities is investigated, two rounds of cultures, at least, being made from nose and fauces. Similar cultures should also be made from the nurses. Those in whom diphtheria bacilli are found should be removed to an isolation ward and appropriately treated. When this method is adopted, a ward can usually be safely considered free from infection after only a few days quarantine.

Attention has been directed to the importance of an examination of the nurses and of a supervision over the toys (especially with regard to the slates and mouth instruments, so frequently supplied by parents), because in several examinations in our wards, in connection with cases of post-scarlatinal diphtheria, these have appeared to be important factors in stamping out infection" (Pugh, p. 311.)

Large many-bedded wards and common recreation rooms tend to favour the spread of secondary diphtheria, since infected children are in both cases brought into intimate contact with numbers of more or less susceptible patients.

"The possible harm which may be done by the introduction of virulent diphtheria bacilli into a ward is to be gauged, not by the number of patients who develop post-scarlatinal diphtheria, but by the number infected by the bacillus. The former, which alone is recorded in a hospital's statistics, is no guide to the amount of evil which may possibly result from the discharge to their homes and schools of children who, though apparently healthy, carry with them the virulent bacillus of diphtheria."

For this reason the use of prophylactic doses of antitoxin in patients exposed to infection without adequate bacteriological examination, as suggested by W. R. Smith (1900), cannot be recommended, since it encourages a free distribution of virulent bacilli amongst the protected patients, who on their discharge may work havoc amongst others.

CHAPTER XIII.

DIPHTHEROID ORGANISMS IN THE INSANE.

The occurrence of diphtheroid organisms in general paralytics in the mouth, nose, genital organs, and internal organs. Characters of the organisms. Eyre and Flashman's observations. Diphtheria bacilli in the tissues.

WITHIN the last few years Robertson and his collaborators have brought forward evidence to show that diphtheroid organisms, which they consider to be non-virulent diphtheria bacilli, are to be found in the tissues of patients who have died of *General Paralysis of the Insane.* They have also demonstrated similar organisms in certain situations in living persons suffering from various stages of the disease. As a result of their observations, Robertson, McRae and Jeffery (v. 1903) "have advanced the hypothesis that general paralysis is the result of a chronic toxic infection from the respiratory and alimentary tracts, permitted by the general and local impairment of the defences against bacteria, and dependent upon the excessive development of various bacterial forms, but especially upon the abundant growth of a diphtheroid bacillus, which gives the disease its distinctive character" (Robertson, 24. x. 1903, p. 1065). Though the final verdict on the relationship of this organism to the disease must rest on the evidence of more extended observations, the results of the experiments already made are extremely interesting and important, and are briefly summarised below.

Although previously to 1903 several observers had investigated bacteriologically the blood, cerebro-spinal fluid and urine of general paralytics, none had met with organisms which they thought bore any relation to the disease. Early in 1903 Robertson, McRae and Jeffery (v. 1903) published a detailed account of their observations on the

secretions, blood and tissues of general paralytics. Further contributions on the same subject have been published by Robertson (VII. 1903, 24. X. 1903, and 25. X. 1905), Robertson and McRae (V. 1905), and Robertson and Shennan (IV. 1903), on which the following account is based.

The occurrence of diphtheroid organisms in general paralytics.

(1) *In the mouth and throat during life.*

"We have succeeded in obtaining cultures of the Klebs-Loeffler bacillus from the tonsils, pharynx, carious teeth and saliva of cases of general paralysis during life, and have also observed the organisms in considerable numbers in film preparations made directly from material from their surfaces. In the only instance in which we have been able to obtain expectoration for examination the Klebs-Loeffler bacillus was found to be present in enormous numbers."

In many other instances, however, they failed to find diphtheria-like bacilli in these situations, and Robertson clearly states that he does "not attach any special importance to the occurrence of the organisms in the throat."

(2) *In the genital organs during life.*

Robertson and McRae (V. 1905) state that apparently all female genital paralytics suffer from leucorrhoea (36 consecutive cases). In all 14 of such cases bacteriologically examined he obtained diphtheroid organisms in smears and in cultures, and he also found them in material taken from the surface of the urethra of 22 consecutive cases of general paralysis in the male.

(3) *In the blood during life.*

Nine observations were made of the blood during life, but diphtheroid organisms were never found.

(4) *Cultures from the tissues after death.*

Twenty cases altogether were examined as recorded in the first paper (V. 1903), and from the tissues of 17 diphtheroid bacilli were obtained in cultures and "in the three from which they were not isolated they were readily found in microscopic preparations of the stomach in two of the cases and in a film preparation of the material on the surface of the trachea in the third."

The following table shows the number of times the organisms were isolated from different situations.

Situation	Cases examined	No. of cases in which diphtheroid organisms were isolated
Tonsils or pharynx	12	9
Bronchi	5	3
Lung tissue	6	3
Stomach	16	7
Inflamed portion of ileum	16	5
Brain	16	4
Bone marrow	5	0

In two later examinations Robertson and McRae (v. 1905) obtained these organisms from patches of localised purulent cerebral meningitis in two cases of general paralysis. In one case they were associated with *B. coli* and in the other with pneumococci.

"The tonsils were almost constantly found to contain small purulent foci on section, and it was chiefly from these that the growths of the diphtheria organism were obtained."

The method of obtaining the cultures from the stomach and intestines was as follows: "Cultures were made from the submucous or mucous coat of the small intestine and stomach, after searing the outer surface and incising the peritoneal and muscular coats with a sterile knife, from the inner surface of these organs."

These cultivations were made on byno-haemoglobin agar.

(5) *Histological evidence of the presence of diphtheroid organisms in the tissues of general paralytics.*

Robertson (VII. 1903) studied the morbid alterations in the alimentary tract of 40 cases of general paralysis, and sought for the presence of the diphtheroid bacillus in 20. Chronic catarrhal changes of a severe nature were constantly found, either in the stomach or small intestines, and frequently in both.

The respiratory tract was studied in five cases. For the purpose of identifying the bacilli he used a modification of Neisser's method, and Weigert's modification of Gram's method. Working with these and other methods he was able "to recognise in the catarrhal exudations in the alimentary or respiratory tracts of all the 20 cases investigated, a bacillus identical in form and staining reactions with the organism isolated by culture methods. In most of the cases the bacilli were to be observed in considerable numbers, and in eight they were present in

very great numbers. In these eight cases their ascertained distribution was as follows:—In the stomach in three cases; in the stomach and lower part of the ileum in one case; in the crypts and surface of the tonsils in two cases; and in the bronchi and stomach in two cases. The organism was found in the respiratory tract in each of the five cases in which this region was examined."

He states that in one of his cases the bacilli were so abundant in the lower respiratory tract that in a transverse section of one of the bronchi stained by Gram's method, in which microscopic examination proved that only those organisms had retained Gram's stain, a broad violet ring lying on the surface of the mucous membrane could be seen with the naked eye.

He also observed a filamentous organism in the lymphatics of the walls of the alimentary and respiratory tracts. "This organism has been observed in enormous numbers in five of the 20 cases investigated, namely, in two cases in the wall of the stomach alone, once in the stomach and ileum, once in the ileum alone, and once throughout the whole extent of the bronchi and trachea." "This organism appears as smooth threads of various lengths, and, as seen in carbol-thionin preparations, shows alternate pale and dark portions, the latter being shorter. Bacillary forms are common." The author gives some reasons for thinking that the organism just described may be a streptothrix form of the diphtheroid organism.

(6) *Characters of the organism.*

The organism grows well on serum and byno-haemoglobin agar producing colonies identical with those of the diphtheria bacillus. Morphologically it is indistinguishable from the diphtheria bacillus and shows well marked polar granules by Neisser's method. Acid is rapidly produced in glucose broth. The other cultural characters have not been described.

Guinea-pigs are not affected by subcutaneous or intraperitoneal injections. Intrapleural injection into a white rat caused death in five days, and "microscopic examination of the tissues showed that the organism had multiplied at the seat of injection and spread into the adjacent pulmonary tissues and into the pericardium." Three rats were fed with bread mixed with broth cultures and remained well for three or four weeks. After this time they began to show morbid symptoms which increased until they became acutely ill. At first they showed

slowness and unsteadiness in gait and drowsiness after feeding. "Later they manifested distinct motor weakness, marked incoordination of movement, dyspnoea, great drowsiness, and looseness of their motions." One was killed when moribund and the others died. Control animals under identical conditions remained healthy.

Microscopical examination of these rats revealed a series of pathological changes, differing in individual cases only in degree. Gastro-intestinal catarrh, localised particularly at the upper part of the intestine, proliferative and degenerative changes of the liver, and inflammatory changes of the lungs were found. The most interesting changes were observed in the brain and consisted of extensive degeneration of a large proportion of the cortical nerve cells, early acute periarteritis, proliferation of the neuroglia and mesoglia cells, and infiltration of the pia-arachnoid.

During the course of several months Dr C. L. Bruce from time to time subcutaneously inoculated a goat with cultures of this diphtheroid organism in order to obtain an immune serum. After a time the animal developed signs of a severe alimentary disturbance. "It became tottering in its gait and about six months from the time the last subcutaneous injection had been made it had a seizure closely resembling the congestive attack of a general paralytic. It rallied to some extent, but died a few days later." Cultures of the diphtheroid bacillus were obtained from the oesophagus, and the brain showed changes resembling those found in general paralysis.

Robertson and Shennan (1903) summarise the results of their animal experiments as follows:—"Whatever the relation of this organism may be to general paralysis of the insane, the evidence we have obtained distinctly shows: (1) that when introduced by way of the alimentary tract in the form of broth cultures, it is capable of producing in the rat a series of morbid phenomena, which especially affect the nervous system, and which when once established may go on progressively till death results, even though the feeding with cultures is stopped; and (2) that the associated changes in the central nervous system have a distinct resemblance to those which occur in dementia paralytica."

Some of the work of the investigators, who have just been quoted, was repeated by Eyre and Flashman (1905). They examined by means of cultures on serum material from the throats of 138 insane patients, of whom 60 were suffering from general paralysis, and 78 from other forms of insanity, and found the diphtheria bacillus in seven, and

Hofmann's bacillus in 14. The diphtheria bacilli occurred in the throats of three (5 %) of the 60 general paralytics.

The injection into guinea-pigs of small quantities of living cultures or of the filtered products of two of these three strains was without effect. One of these strains, but not the other, was capable of killing guinea-pigs when the living bacilli were injected in large doses.

A series of examinations by means of cultures was also made from material derived from autopsies on 10 cases of general paralysis and 26 cases of other forms of insanity. Swabbings from the pharynx and bronchi, scrapings from the mucous membrane of the intestine, and specimens of the cerebro-spinal fluid, heart's blood and bile were obtained. Diphtheroid bacilli were never found in the cerebro-spinal fluid, or the bile. In the examinations of general paralytics diphtheroid bacilli were found in four out of seven cultivations from the pharynx, three out of ten from the bronchi, one out of eight from the heart's blood, and one out of ten from scrapings from the intestinal mucosa. No further investigation of these organisms appears to have been made. Diphtheroid bacilli were also met with on four occasions in the respiratory tract in 26 examinations of patients suffering from other forms of insanity. The authors are satisfied that not one of the organisms isolated from post-mortem material was *B. diphtheriae* (Eyre, 9. XII. 1905, p. 1558).

As a result of their experiments these authors came to the conclusion that they were "unable to trace any causal connection between *B. diphtheriae* and general paralysis of the insane," and suggested that Robertson and his colleagues were dealing with a "local or Asylum infection."

Local conditions might undoubtedly account for the high percentage of infection in various parts of the buccal cavity observed by Robertson, and the fact that diphtheria-like organisms are to be found both in vagina and male urethra has been pointed out by several observers (see p. 378). The extraordinary percentage of infection of the genito-urinary organs in Robertson's series, however, suggests that the presence of these organisms was more than accidental. His observations on their occurrence in large numbers and in every case in the inflamed portions of the gut, their frequent occurrence in the brain and their action on animals, all point to their connection with the disease, and render further investigations desirable. Eyre (9. XII. 1905) states that in his ten cases no catarrhal areas were discovered in the intestinal canal,

although a careful search was made in each case. Eyre and Flashman (1905) only examined the cerebro-spinal fluid and not the brain, and appear to have made no animal experiments comparable with those of Robertson and Shennan.

In connection with this subject certain records of the finding of diphtheria-like bacilli in the brain and blood, and in the crypts of the tonsils of persons who had not suffered from diphtheria, and certain observations on the fate of diphtheria bacilli in the alimentary tract, are of interest.

Johnston and Goodall (1902) cultivated from the heart's blood, but not from the cerebro-spinal fluid, of a woman, who died in "an acute confusional state, possibly a phase of dementia paralytica," a diphtheria-like bacillus, whose action on animals was not tested.

Head and Wilson (1899) cultivated diphtheria bacilli, virulent for guinea-pigs, from the brain of a suspected case of rabies, and Morrell and Wolf (1906) twice isolated virulent diphtheria bacilli during life from the cerebro-spinal fluid of a child suffering from general miliary tuberculosis and tubercular meningitis. Although no signs of diphtheria were present diphtheria bacilli were also found in the throat. Howard (1894) isolated non-virulent diphtheria bacilli from the heart valves of a patient who died of acute ulcerative endocarditis.

Marzinowsky (1900) examined by means of cultures both the surfaces and the crypts of the tonsils of 15 persons, who had died from various diseases.

Non-virulent diphtheria-like bacilli, retaining Gram's stain and showing polar granules by Neisser's method, were isolated from the crypts in six cases (20 %), but only in one instance from the surface of the tonsil. In two other cases (pneumonia, anthrax) virulent diphtheria bacilli were found in the crypts. Schütz appears to have isolated both attenuated diphtheria bacilli and diphtheria-like bacilli from the sputum of tuberculous patients.

Although several examples of diphtheritic lesions of the stomach have been recorded (p. 97), but little attention has been paid to the fate of diphtheria bacilli in the intestinal canal. Süsswein (1902) has recorded a few observations on this subject. In 15 cases of diphtheria examined during life, he was unable to detect the diphtheria bacillus in either the gastric or intestinal contents. At the autopsies on eight cases he was able to detect the diphtheria bacillus in films from the gastric contents in four cases, but was able to obtain cultures in two

cases only. He never observed it in the jejunum. Diphtheria bacilli kept at 37° C. in acid "stomach contents" died within an hour, but neither neutral "stomach contents" nor the contents of the gall-bladder affected them.

Schoedel, J. (1900) apparently observed diphtheria bacilli both in the lower ileum and in the freshly passed faeces of persons suffering from diphtheria.

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