THE RESULT OF 950 BACTERIOLOGICAL EXAMINATIONS FOR DIPHTHERIA BACILLI DURING AN OUTBREAK OF DIPHTHERIA AT CAMBRIDGE AND CHESTERTON.

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The following is an account of the bacteriological examinations for diphtheria bacilli made by me at the University Pathological Laboratory, from October 23, 1900 to Jan. 5, 1901; a period which includes all but the first week of the outbreak described in the preceding paper.

The number of persons examined was 692. Some of these having been tested several times, the total number of examinations made exceeded 950.

Among the 692 persons examined, there were 42 notified cases of diphtheria[1]. Besides these there were about 22 other notified cases in the town which were either sent direct to the hospital without being examined by me, or had been taken ill before I began my work.

Bacteriological examinations for diphtheria bacilli in the throats and noses of suspected cases of diphtheria, and those who have been exposed to infection require to be at once rapid and accurate. Valuable as these examinations undoubtedly are, it must be admitted that they are attended with some difficulty, and in unskilled hands have given rise in the past to not a little inconvenience and pecuniary loss. They have consequently become looked upon with some distrust, not only by a portion of the general public, but also by not a few medical practitioners. The frequent occurrence of the pseudo-diphtheria bacillus of

[1] Including one from a neighbouring village, and excluding four cases in which symptoms of diphtheria were absent or uncertain and which were notified because suspicious bacilli—which were afterwards recognised as Hofmann's bacilli—had been found.

Hofmann[1] in cultures from the human pharynx and nose is the chief source of difficulty[2]. It is true that there are two reliable tests which serve to distinguish this bacillus from the bacillus of diphtheria, namely the absence of acid-formation in glucose media, and the absence of pathogenic power, particularly the power to produce inflammatory oedema at the seat of subcutaneous inoculation in guinea-pigs. But each requires time, and the opinion of the bacteriologist to be of practical value often cannot await the preparation of pure cultures and the injection of animals,—to say nothing of the expense of such investigations when carried out on the large scale which is often desirable. What then is to be done? The answer I believe to be that it is possible to train the eye to distinguish, with a sufficient degree of precision, the diphtheria bacillus from the bacillus of Hofmann. But I think that the eye cannot become sufficiently trained for this purpose unless the observer frequently tests the opinions which he forms on morphological grounds, by isolating his cultures and testing them in various ways, including the injection of animals. Such tests should never be omitted in cases of doubt or when much depends upon the issue. Further the making of drawings with the aid of the camera lucida is of the greatest assistance in training the eye, because it concentrates the attention upon individual bacilli for a far longer time than would otherwise be the case, and because it gives the observer the opportunity of comparing his

[1] The pseudo-diphtheria bacillus first described by Löffler, and afterwards by Hofmann and others, is called throughout this paper by the name of Hofmann, in order to clearly distinguish it from the true diphtheria bacillus with which the name of Löffler is usually associated.

[2] Roux and Yersin (*Ann. de l'Inst. Pasteur*, 1890, vol. IV. p. 385), ten years ago expressed the opinion that the frequent presence of the pseudo-diphtheria bacillus in the mouths of healthy persons and others not suffering from diphtheria, does not interfere with the bacteriological search for the true bacillus. They arrived at this opinion because of the small number of colonies of the "pseudo-" bacillus when present. It is doubtless true as a rule that the colonies of this micro-organism are not numerous. But I have several times found cultures from the nose and pharynx consisting of very numerous colonies almost all of which were made by the bacillus of Hofmann, and on the other hand from cases of diphtheria both active and convalescent I have had cultures with only one or two colonies of diphtheria bacilli. Roux and Yersin stated that their pseudo-bacilli produced acid, but their description would seem to include the Hofmann bacillus. They wrote before the importance of the acid test had been recognised, and it is probable from the frequency with which they found their pseudo-diphtheria bacilli (in 40 per cent. of the children of a school in a healthy country village) that their group of pseudo-diphtheria bacilli included both the attenuated diphtheria bacillus and the bacillus of Hofmann.

observations with one another. All these means have been used in the investigation with which this paper deals.

The chief interest of the investigation centres in the large number of children of school age examined, coming from four schools, which in varying degrees had been invaded by the disease. These may be arranged as follows

		Number examined.
(1)	Park Street School (14 cases of diphtheria with 4 deaths) ...	51
	Young brothers and sisters of the Park Street School scholars (5 cases)	46
(2)	King Street School (2 cases)	59
	Young brothers and sisters of the above	33
(3)	Occupation Road School (2 cases)	57
	Young brothers and sisters of the above	32
(4)	Romsey Town School and the young brothers and sisters of the scholars (0 cases). No cases of diphtheria occurred in the part of the town in which this school is situated	43
		321

Methods.

The nutrient medium used was alkalised serum to which 1 % of glucose had been added[1]. Horse-serum was used by preference, but owing to the large demand a good deal of ox-serum had to be used as well. Both media proved convenient and reliable. The earliest appearance of a visible growth of diphtheria bacilli was observed in six hours, and the latest in three days. In the latter case the delay was thought to have been caused by the application of a weak carbolic acid solution to the throat previous to the application of the swab[2]. The cultures were examined the day after they were sown and if no growth of any kind had appeared they were re-examined on several days next following.

For staining Löffler's methylene-blue was used diluted with 2 or 3 volumes of water, the cover-glass preparations being mounted in the staining-fluid. The groups of bacilli were observed to decolorise the fluid around them, and they consequently appeared as if mounted in a colourless fluid. This method was selected on account of its quickness, and because specimens so mounted seemed to me to present a more characteristic appearance than if they are washed and dried after staining and mounted in Canada balsam. It was found practical to make 4 to 9 separate preparations on one cover-glass from the different colonies of a single culture-tube. The examinations thereby gained in thoroughness and took less time.

[1] First suggested by Prof. Lorrain Smith of Belfast. For the preparation of this nutrient medium see *Brit. Med. Journ.* 1894, vol. II. p. 1177.

[2] Experience gained during this investigation confirmed the generally accepted opinion that antiseptics applied to the throat not unfrequently render the bacteriological examination useless or misleading. Practitioners can scarcely be reminded too often of this fact.

Cover-glass preparations from *broth* cultures were, before being stained, dipped in 5 % solution of acetic acid for ten seconds, and washed in water to which a few drops of ammonia had been added to more rapidly remove the acid.

When bacilli more or less resembling the diphtheria bacillus were found, the fact was notified to the physician, if any was in attendance, and the microscopic preparation preserved for future reference. Steps were taken to obtain a pure culture for further investigation, but owing to the pressure of work during the early days of the investigation when the number of tubes to be examined daily was very great (on one occasion exceeding 100), the attempt to get a pure culture was in some instances omitted or failed. Nevertheless of 250 cultures of more or less suspected bacilli, over 100 *were isolated* and tested on animals.

The suspicious bacilli found were provisionally classified on the result of a microscopic examination into: (A) long bacilli, microscopically identical with the diphtheria bacillus, and (B) and (C) shorter bacilli which differed from, but had more or less resemblance to the diphtheria bacillus.

When pure cultures had been obtained the bacilli were tested for their power to produce an acid reaction to litmus, when grown for 48 hours in broth containing 1 % of glucose and having an initial alkalinity to litmus equal to about 7 c.c. of normal alkali per litre.

This was followed by injection of broth cultures into guinea-pigs.

The quantity injected was regulated by the reaction shown by the glucose-broth culture; thus, if this had been acid, 0·1 c.c. was the quantity usually employed, if alkaline 2·0 c.c. In a few instances the smallest dose injected of a culture which had shown itself capable of producing acid was 0·5 c.c. The number of injections in the case of each culture, the dose and age of the culture, and the size of the guinea-pigs used are shown in Table III. In all cases the cultures injected were grown in sugar-free broth.

Autopsies on the animals were made in nearly all cases, and since the subcutaneous oedema at the seat of inoculation, the excess of fluid in the pleural cavities, and the reddening of the suprarenals, are so characteristic of death from diphtheria, it was not thought necessary as a rule to practise the injection of culture plus antitoxin.

A description was kept of the naked eye and microscopical appearances of all cultures on serum and in glucose and sugar-free broth. The microscopic preparations of all were kept, and in some instances the serum cultures were sealed with the blowpipe and preserved for reference. When the stress of work became gradually relaxed, drawings were made with the aid of a camera lucida of bacilli from serum, glucose broth, and the sugar-free broth culture injected into the guinea-pigs. And this was done as a routine measure during the latter half of the investigation.

The Morphology of the Diphtheria and Pseudo-diphtheria Bacilli.

The range of variation in form of the diphtheria bacillus is unfortunately great. Wesbrook, Wilson and McDaniel[1] have recently drawn attention to this, and have published drawings of 19 different shapes which this micro-organism may assume. Briefly they recognise three main types, which they call (1) granular, (2) barred and (3) solid colour forms. The last-mentioned contain ("Type D^2") bacilli which generally present the appearance of pairs with opposing extremities flattened and thickened, the distal extremities bluntly pointed or abruptly rounded. Whether the colourless part between the two opposed members of the pair is an actual space between two bacilli or an unstained part of one bacillus they are unable to state. They consider that "these bacilli are probably included under the pseudo-diphtheria or Hofmann group of other observers." Yet in spite of their innocent appearance they were the most prevalent form in an outbreak of diphtheria at Owatonna examined by Wesbrook, and are occasionally the only form present in clinical cases, and frequently pathogenic to guinea-pigs. Prof. Sims Woodhead tells me that he has met with very virulent bacilli of similar appearance. During the recent outbreak here they were never observed.

If I may be permitted to go once again over familiar ground I shall venture to describe briefly the various types of diphtheria and pseudo-diphtheria bacilli, as they have appeared to my eye during my recent observations.

To begin with the pseudo-diphtheria bacillus. A micro-organism which I take to be the pseudo-diphtheria bacillus of Löffler and Hofmann, very common in the pharynx and nasal cavities among the poorer classes in Cambridge, which forms no acid in glucose culture media, and causes no oedema in the guinea-pig, has the following appearance under the microscope. From young serum cultures it appears with considerable regularity as a darkly staining oval bacillus of somewhat variable length, with one (or rarely more) narrow unstained septum. These bacilli present a very characteristic appearance and do not at all closely resemble the common adult forms of the true diphtheria bacillus. Occasionally, however, colonies are met with which contain a fair number of bacilli with several septa, and the differential diagnosis is then more difficult (see Pl. V., Figs. 8,

[1] *Trans. of the Assoc. of American Physicians*, 1900.

11 and 12). In broth cultures I think this bacillus more closely resembles the diphtheria bacillus. Here too the oval bacillus with one unstained septum usually predominates, but giant forms which closely resemble the diphtheria bacillus are generally present also and may be numerous. They are many times the length of the oval bacillus, often clubbed, and are, I think, rather thicker than the diphtheria bacillus (see Pl. V., Fig. 4). It is cultures in which these giant forms predominate which present the closest resemblance to diphtheria bacilli.

The true diphtheria bacilli in young serum cultures vary greatly. Short forms like those just described were frequently seen, but I have not yet found them the only forms present in a virulent culture. I regard them as young forms. Like Wesbrook I found that the commonest type of diphtheria bacillus in original serum cultures was the granular or, as I prefer to call it, the *beaded bacillus*. This is a faintly staining curved bacillus of irregular width, usually two or three times the length of Hofmann's bacillus, with one or more darkly staining rounded dots, often terminal, sometimes central (see Pl. III., Figs. 6 and 10, and Pl. IV., Fig. 7). Next in frequency was the *barred or segmented bacillus*. It varies in length but is always longer than Hofmann's bacillus, is curved and of irregular thickness. It stains darkly with several narrow unstained or faintly stained linear intervals. One of the roughly rectangular segments is often larger than the others (see Pl. III., Fig. 5). If the enlarged segment is terminal the bacillus is club-shaped, if mesial spindle-shaped.

Intermediate between these types were the "streptococcus forms" (Pl. III., Fig. 3) which may appear as a row of dots at regular intervals in a pale bacillus. If faintly stained the dots alone may be seen and the bacilli may indeed be taken for streptococci; but the regular and somewhat stiff curve which they assume, and the uniformity of their length and finally their 'arrangement' will suggest their true nature, and in case of doubt re-staining more darkly will remove all difficulty.

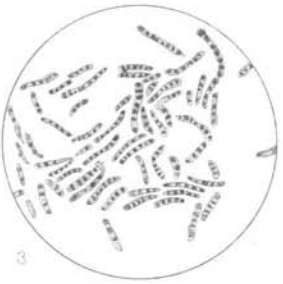
Some cultures were formed of bacilli so uniformly stained that it was difficult to see any trace of segmentation and granulation (Pl. III., Fig. 1). Thus it will be seen that I learned to recognise five types of diphtheria bacilli from young serum cultures,

(1) Oval bacilli with one unstained septum. Young forms.
(2) Long, faintly stained, irregularly beaded bacilli.
(3) Regularly beaded bacilli. Streptococcal forms.
(4) Segmented bacilli.
(5) Uniformly stained bacilli.

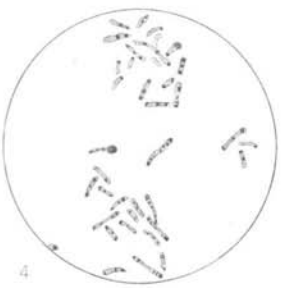
I. Diphtheria bacilli, virulent, acid formers.

B.C. 792. 28 . xi.

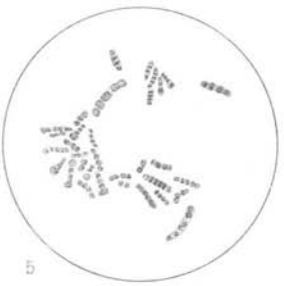
B.C. 792. 3 . i.



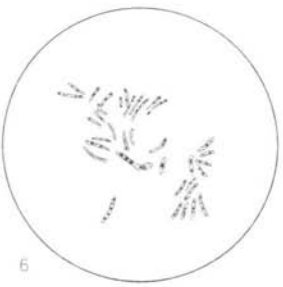
Mrs M. 250.



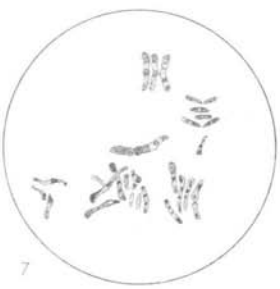
M.Cl. 723. 1 . xii.



M.Cl. 723. 17 . xii.



M.Cl. 723. 20 . xii.



M.Cl. 723. 1 . xii.
broth. 3 days.

M.Cl. 723. 1 . xii.
Glucose broth. 24 hours.

G.Gd.

R.G. 768.

R.G. 768.
Serum Subculture. 24 hrs.

R.G. 768.
Serum Subculture. 24 hrs.

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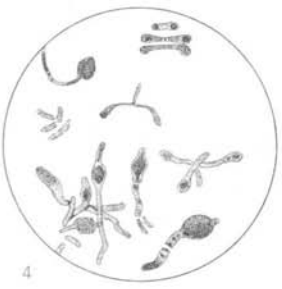
Diphtheria bacilli (continued)

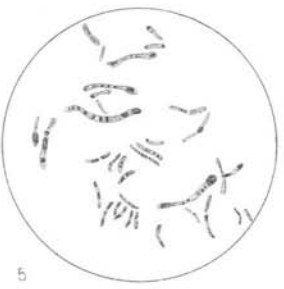
V.Th. 734
3 days.

V.Th. 734
Serum Subculture. 2 days.

G.N. 1. a. Chr. Fibrinous Rhinitis.
Broth. 2 days.



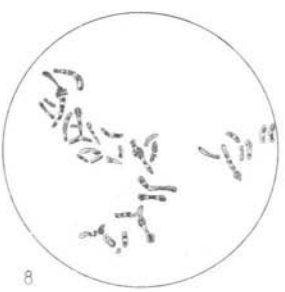
V.Th. 734.
Glucose broth. 2 days.



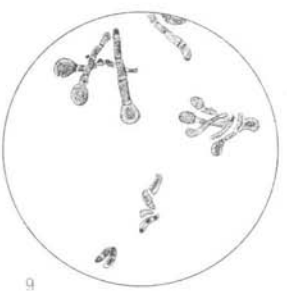
V.Th. 734.
Sugar-free broth. 2 days.

G.D. 714.
Glucose broth. 3 days.

G.D. 714.
3 days.



G.D. 714.
Sugar-free broth. 24 hours.

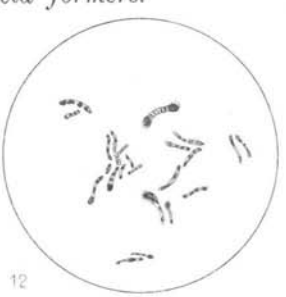


G.D. 714.
Glucose broth. 24 hours.

II. Non-virulent diphtheria bacilli, acid formers.

A.G. 721. 3 days.
During convalescence from diphtheria.

A.G. 721.
Sugar-free broth. 24 hrs.



A.G. 721.
Glucose broth. 24 hrs.

E. Wilson, Cambridge.

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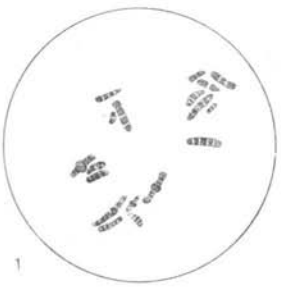
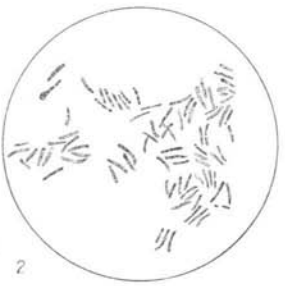
Non-virulent diphtheria bacilli (continued).



Mrs C. 793.

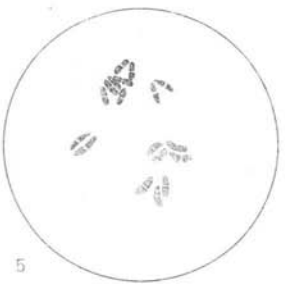


B. 782.
Sugar-free broth. 8 days.

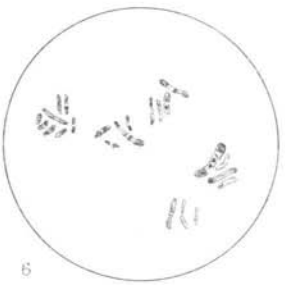
B. 782.
Sugar-free broth. 24 hrs.

III. Pseudo-diphtheria bacilli, non-acid formers.

GG. 375.
Glucose broth. 2 days.

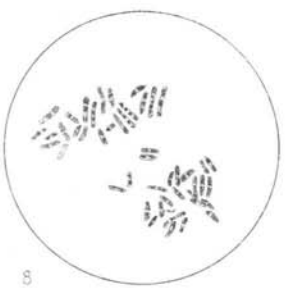


D.Cl. 733.

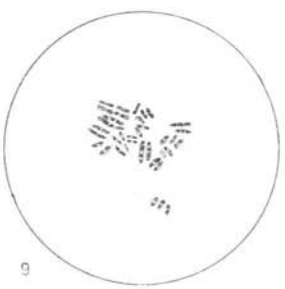


D.Cl. 733.
Glucose broth. 2 days.

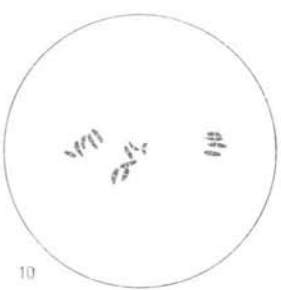
M.W. 777.



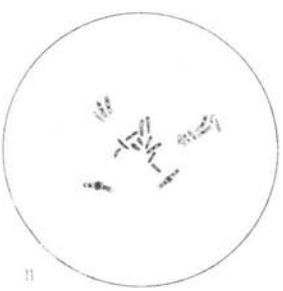
G.D. 754.
3 days.



R.A. 796.
Glucose broth. 24 hrs.



A.L. 789.
Sugar-free broth. 2 days.



M.Cl. 723. 3 days.

M.Cl. 723. 3 days.

4th Culture from this case, the previous three being diphtheria bacilli (see Table II.).

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In general the characteristics of the bacillus other than beading and segmentation were that they were more or less curved and of varying thickness. The "arrangement" varies; occasionally the bacilli resembled a lot of pine needles on the ground. Broth cultures were apt to be much clumped. In any one culture the bacilli, if we exclude what I take to be young forms, conformed fairly closely to one type, and one could often recognise the same type in successive cultures from the same person. On glucose broth, when it had become acid, extraordinary forms sometimes appeared with one enormously swollen segment either at the end or in the middle. These very long forms with a swollen extremity resembled serpents, while the shorter forms with a more central swelling were something like elongated peg-tops (see Pl. IV., Figs. 4 and 9).

When once one had become well acquainted with its range of variation it was fairly easy to recognise the diphtheria bacillus and to distinguish it from all others (that is, if the acid-producing but non-virulent bacillus which resembles it in all other ways be admitted as an attenuated diphtheria bacillus). The bacillus of Hofmann was the only one which presented any difficulty, and about this I slowly became persuaded that it could as a rule be excluded on morphological grounds alone. This of course would not have been the case had I met with the short Hofmann-like yet virulent diphtheria bacillus described by Wesbrook, but it did not occur, and it is worthy of note that 69 cultures classed provisionally under B or C (bacilli morphologically resembling Hofmann's bacillus) were isolated and tested, with the result that not one produced any acid in glucose broth or caused oedema in the guinea-pig[1]. This result was somewhat unexpected, for I had anticipated that among them would have been found some true diphtheria bacilli. But this was not so, and the investigation of the pure cultures which were isolated showed that all the true diphtheria bacilli among them, together possibly with four Hofmanns[2], had been

[1] It is true that 8 out of 83 animals injected died within 10 days of inoculation, but that they did not die from the bacillus in question is clearly shown by the following considerations, (*a*) there was no oedema at the seat of inoculation in any during life, (*b*) nor after death, and there was no excess of pleural fluid, (*c*) the injections were in all cases repeated, sometimes in larger doses, and the animals remained well, (*d*) though the 83 injections occupied two months and more, all deaths happened during one week, and were probably therefore due to some cause other than the injection.

[2] In these four instances it seemed probable that the bacilli first seen and classed under A were true diphtheria bacilli, and that the attempt to isolate them failed and resulted in isolating a pseudo-diphtheria bacillus instead.

classified on microscopical grounds under A, and that classes B and C contained none but the non-acid-forming pseudo-diphtheria bacillus.

These harmless bacilli, which I take to be the pseudo-diphtheria bacilli of Löffler and Hofmann, were as I have said easily distinguished from the true diphtheria bacilli found during this epidemic when grown on alkalised serum. Their colonies on serum usually became after a few days larger and whiter than those of the diphtheria bacillus but there was often little or no difference at the end of 24 hours, and in other respects in their mode of growth in broth and on gelatine they closely resembled the true diphtheria bacillus, except that they grew more quickly. They did not produce a definite film on broth, but it is rare for diphtheria bacilli to do so until they have become well accustomed to the medium.

The Distribution of the Diphtheria and Pseudo-Diphtheria Bacilli among those examined.

692 persons were examined, including 42 notified cases of diphtheria. Among these 42 notified cases no suspicious micro-organisms were found in 6. From 5 Hofmann's bacillus unassociated with the diphtheria bacillus was isolated and proved to be incapable of producing acid, or causing oedema in guinea-pigs. The diphtheria bacillus was found in 24 (57 %)[1]. From 16 of these 24 cases 20 pure cultures were isolated, and all but one proved to be highly virulent. In this latter instance the bacillus which was morphologically identical with the virulent diphtheria bacillus, and formed acid in glucose broth, was obtained from a patient examined for the first time during convalescence from diphtheria. From the remaining 7 cases bacilli more or less closely resembling the short diphtheria bacillus were found, and classified provisionally under B. These were not tested on animals but from subsequent experiments with similar cultures I should say that the majority were probably Hofmann's bacilli.

Among the 650 other persons, bacilli morphologically identical with the diphtheria bacillus were found in 19. From 8 of these 19 persons 9 pure cultures were obtained. All produced acid and all but three

[1] Among over 5000 suspected cases of diphtheria in New York Park and Beebe found the diphtheria bacillus in about 60 %. Morse found it in 72 % of 301 cases of diphtheria in the Boston City hospital. (Cited by Welch, "Bacteriological Investigation of Diphtheria in the United States," *Am. Journ. of Med. Sci.* Oct. 1894.)

were highly virulent. These latter were entirely without virulence. In addition to these, four attempts, previously mentioned, to isolate a diphtheria bacillus which had been recognised as such on the strength of a microscopic examination of the original culture, failed, and only the bacillus of Hofmann was obtained (proved in all these cases to be non-virulent). It may be that Hofmann's bacillus was mistaken for the diphtheria bacillus at the first examination, but I think it more probable that both the true and the pseudo-diphtheria bacillus were originally present and attempts to obtain a pure culture of the former resulted in isolating the latter only.

Of these 19 persons, who were not notified cases of diphtheria, and in whose throats diphtheria bacilli (proved to be virulent in five cases) were found, a few had slight sore throat at the time of examination, and all but one received injections of antitoxin. Without this some would doubtless have developed into clinical cases of diphtheria. It is therefore impossible to say how often diphtheria bacilli were found in healthy persons. But this much may be stated that *diphtheria bacilli were found only in actual cases of diphtheria, or among those who had come directly into contact with such cases.* These latter were either children attending the school most affected, or inmates of houses where there was an actual case. Among a very large number of other people examined the diphtheria bacillus was found not once.

The Hofmann's bacillus was found altogether 157 times, and isolated in pure culture 69 times. All these 69 cultures failed to produce acid in glucose broth, and caused no local oedema in guinea-pigs. It 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 ordinary schools where there was little or none[1]. Among the small number of children of the upper classes examined it was conspicuous by its rarity.

The 25 cultures isolated which proved to be virulent, killed guinea-pigs of 200—500 g. in two or three days, when 0·1 c.c. of a 48 hours' broth culture was injected. (A few of the earlier experiments were made with older cultures and in four instances the smallest dose given was 0·5 c.c.) In three instances 0·1 c.c. of a 48 hours' culture failed to kill, but these cultures were very poorly grown, and when the

[1] Roux and Yersin have made a similar observation.

injection was repeated a few days later, after the culture had passed through one or more generations in broth, and had become more accustomed to this medium, death took place within forty-eight hours. It may therefore be stated that between the cultures which killed in doses of 0·1 c.c. and those which were entirely harmless in doses of 2·0 c.c. there were none with intermediate degrees of virulence. The ratio of non-virulent diphtheria bacilli to virulent diphtheria bacilli found was 4 : 25, or 16 %. Even if these non-virulent diphtheria bacilli be entirely harmless to man and incapable of becoming virulent, this relative frequency of occurrence would not seriously impair the value of the bacteriological test. The case of Hofmann's bacillus is quite different unless it be excluded, for it occurred six times as often as the diphtheria bacillus, or in about 23 per cent. of the healthy people examined.

From the above account it will be seen that the bacilli isolated and tested may be classified as follows[1].

1. Bacilli, identical in appearance both in culture and under the microscope with the diphtheria bacillus.

(a) Pathogenic acid-producers = virulent Klebs-Löffler bacilli 25

(b) Non-pathogenic acid-producers = the so-called attenuated diphtheria bacilli 4

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

Non-pathogenic, non-acid-producers, Löffler-Hofmann, so-called Pseudo-diphtheria bacilli 69

This classification was first made by Park and Beebe[2].

Is the Pseudo-Diphtheria Bacillus of Löffler and Hofmann an attenuated Diphtheria Bacillus capable of becoming dangerous?

This is a most important question, for on its answer depends the whole basis of the measures which should be taken for combating diphtheria. For if the so frequent Hofmann bacillus can under certain conditions, say insanitary environment, become the true diphtheria bacillus, then diphtheria must be combated by general sanitary measures, and it is

[1] During the past six years I have met with other bacilli which more or less resemble the diphtheria bacillus. These mostly came from the skin. The above-mentioned types are the only diphtheroid forms which I have found in the nose or pharynx.

[2] *New York Med. Rec.* XLVI. 1894, p. 385.

impossible to seek out and strictly isolate those who have these micro-organisms in their mouths. On the other hand, if the Hofmann bacillus is entirely harmless, then isolation of those who carry about dangerous bacilli is possible and reasonable and should be strictly enforced, even at the cost of some individual hardship.

Roux and Yersin, Hewlett and Knight[1], Richmond and Salter[2] and others have laid stress on the frequency with which the pseudo-diphtheria bacillus is found in the mouths of persons convalescing from diphtheria, and its relative infrequency during the acute stage of the disease. But surely it is often overlooked in the early stage of the attack because one then discovers the virulent bacillus so easily and does not trouble to look any more. On the other hand when the diphtheria bacilli are disappearing and are hard to find, a long and careful search is made, and the pseudo-diphtheria bacillus previously overlooked is seen for the first time. I have frequently tested on animals these bacilli occurring during convalescence and have always found them to be as completely devoid of virulence as those derived from healthy persons. Roux and Yersin speak of diphtheria bacilli intermediate in virulence between the pseudo and the fully virulent diphtheria bacillus. Such may well exist if the acid-forming non-virulent bacillus is an attenuated diphtheria bacillus. These bacilli of low virulence have not been met with in Cambridge, and the non-virulent diphtheria bacillus was rare. But the bacilli which we are now considering are those which do not produce acid out of glucose. These are in my experience always devoid of virulence, and have never been found to cause local oedema even in relatively large doses (2·0 c.c. of cultures rich in bacilli).

The crucial question is, Can the diphtheria bacillus be converted into the bacillus of Hofmann, and can the bacillus of Hofmann become the virulent diphtheria bacillus? Neither question can be definitely answered. Roux and Yersin starting with virulent cultures of diphtheria bacilli, and growing them under unfavourable conditions of temperature in a current of air, obtained a non-pathogenic bacillus which produced no toxin. Sometimes the change was quick, sometimes slow, and it is significant that they did not produce intermediate degrees of virulence as regularly as in the attenuation of the bacillus of anthrax. In one instance, however, the virulent bacillus became replaced by one which produced marked oedema but did not kill,

[1] *Trans. Brit. Inst. Prev. Med.* first series, p. 7, 1897.
[2] *Guy's Hospital Reports*, vol. LIII. p. 55, 1898.

before its virulence finally disappeared. But this attenuation proves nothing but that the diphtheria bacillus may lose its virulence, which is not denied, but that it should turn into the *pseudo-diphtheria* bacillus is quite another matter. Hewlett and Knight believed that they were able to produce this transformation on one occasion, but attempts to repeat this experience with other bacilli were not so successful. In the one successful instance they reject but they do not exclude the possibility that they may have started with a mixture of the two bacilli.

Attempts to make the pseudo-diphtheria bacillus virulent have been recorded. Roux and Yersin could increase the virulence of a bacillus which caused oedema, though it did not kill, but were unable to give virulence to non-virulent forms. Hewlett and Knight believed that they were able in one or two cases to transform the pseudo-diphtheria bacillus of Hofmann into the virulent diphtheria bacillus. In the one experiment which they record the culture passed through a large number of generations in tubes and plates as well as in animals. For a long time it remained inoffensive, and when it became virulent it did so suddenly and for no better reason than that the 19th and 20th generations were serum cultures incubated at 37° for a week. This acquisition of virulence was associated with the appearance for the first time of the faculty of forming acid. In 1898 Richmond and Salter (*loc. cit.*) published a paper on "The Etiological Significance of the Diphtheria Bacillus and its Variants" in which they briefly stated that they had succeeded in transforming the pseudo-diphtheria bacillus of Löffler and Hofmann into the virulent diphtheria bacillus by repeated passage through certain birds. The transformation was gradual, the bacilli becoming longer, staining differentially with methylene-blue, and ultimately forming acid in neutral broth, and killing guinea-pigs with all the pathognomonic signs of experimental diphtheria. More recently Salter[1] has described in detail the conversion of one of the four Hofmanns which he transformed. The bacillus employed came from a case of post-scarlatinal diphtheria. Morphologically it was a typical Hofmann's bacillus. It produced an acid reaction in broth, and was entirely harmless to guinea-pigs. After its exaltation it was definitely but not strongly toxic for these animals, 5·0 c.c. of a broth culture producing death on the sixth day. The usual gelatinous oedema at the seat of inoculation, pleural exudation and

[1] *Trans. Jenner. Inst. Prev. Med.* Second Series, p. 113, 1899.

reddening of suprarenals was found after death. Its pathogenic action was counteracted by antitoxin.

Salter was moreover able to kill guinea-pigs with mixtures of diphtheria toxin and antitoxin which contained just sufficient of the latter to prevent death, by adding small quantities of filtered culture of (unconverted) Hofmann's bacilli. From this he concluded that diphtheria protoxoid is a common product both of the pseudo-diphtheria and of the diphtheria bacillus.

In view of the wide distribution of Hofmann's bacillus among healthy persons in Cambridge and elsewhere the conclusion arrived at by Richmond and Salter that the pseudo-diphtheria bacillus is an attenuated variety of the true causal agent of diphtheria is, if well founded, of great importance. But until the position of the bacillus of Hofmann has been clearly established, and it has been proved capable of being converted into the virulent diphtheria bacillus, not merely by laboratory procedures, but further, under natural conditions, we must not conclude that the causal agent of diphtheria is widespread, nor weaken in our efforts to find out those who harbour the *virulent* diphtheria bacilli, and to isolate them until they have become freed from these micro-organisms.

There is no evidence that bad drains and insanitary environment can ever convert non-virulent into virulent bacilli and originate diphtheria. Though the influence of climate and season is marked, it has not been shown that it is exerted upon the bacillus rather than upon the human being. All experience goes to show that except in occasional instances, such as where it is distributed by milk, or contracted from animals, cases of diphtheria are attributable to the bacilli being conveyed, in most instances directly, from the one person to another. The following is a good instance of what I believe is the common mode of transmission.

B. C. aged 14, was taken ill with headache and sore throat on December 26th. On the 27th a patch of membrane was seen on the left tonsil. An injection of antitoxin (2400 units in 3 c.c.) reduced his temperature from 101·5° to 97·6° F. within four hours, and removed all the constitutional symptoms. The latter did not return, but a condition of follicular tonsillitis associated with the continued presence of the diphtheria bacillus persisted for many days. A cultivation taken on the 27th gave the diphtheria bacillus. A similar culture was obtained from his sister who remained well.

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 enquiry 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 it easily found its way to his brother, for the two boys slept in the same bed.

The Need of more than one Negative Bacteriological Examination of Patients convalescent from Diphtheria.

The Boston Board of Health, U.S.A., require two consecutive negative examinations of convalescents before they are pronounced free from infection, and for hospital patients the rule is three consecutive negatives[1]. During the recent epidemic here, the medical practitioners have been requested to submit swabs from their convalescent patients until three consecutive negative examinations have been obtained. Table II. shows a number of these consecutive examinations. It will be seen that more than once two consecutive negative examinations were followed by the finding of virulent diphtheria bacilli. It was thought that some of the misleading negative examinations were caused by the swabs being taken too soon after the application of some antiseptic. But it may be that in some cases the diphtheria bacillus lurks in the nasal cavities, or perhaps in one of the sinuses communicating with them, and only occasionally finds its way into the pharynx. The fact that after pharyngeal diphtheria the bacillus has been found in the nose when it was not detected in the pharynx seems to support this suggestion[2].

The need of more than one negative examination is clearly shown by the following history. *P* a scholar attending the school in which diphtheria had broken out and *Q* his baby brother were examined on October 24th, and diphtheria bacilli were found in the boy, but not in the baby. *P* who was staying away from home received a dose of antitoxin and remained well, but *Q* received none. A short time after *P* had returned home *Q* got diphtheria. When he had recovered, another

[1] See paper by H. W. Hill, M.D., *Journ. of the Massachusetts Association of Boards of Health*, vol. VIII., Oct. 1898.

[2] Wolff in 1895 examined bacteriologically the accessory sinuses of the nose in fatal cases of diphtheria etc. The *B. diphtheriæ* was found in 12 out of 22 cases of diphtheria in one or more of these sinuses, including once in the frontal, and six times in the sphenoidal sinus. Cited by Howard and Ingersoll. *Am. Journ. of Med. Sciences.* May, 1898.

examination of the two children was made on November 21st and the result was negative in each case. The physician in charge was satisfied with this single negative result and after this no precautions were taken to isolate the children any longer.

Three weeks to a month later two other boys, *X* and *Y*, fell ill with diphtheria within a few days of each other. One of them (*X*) had been going to the house of *P* and *Q* to have music lessons from the father. It was *Y*, however, who was taken ill first.

It was thought possible that the boy who had the music lessons had got the infection at the house of *P* and *Q*—though he had seen no one there but the father—and had passed it on to his brother, who being perhaps a little more susceptible was the first to fall ill. Consequently another series of examinations of the *P* and *Q* family was made on December 21st, with the result that diphtheria bacilli were found in *P* and *Q*, the mother, and the maidservant. The father and a young sister were the only members of the household free from infection. It is interesting to note that these two alone had never come into contact with the baby when he was ill.

The following table shews the consecutive examinations of the members of this household.

TABLE I.

D = A clinical case of diphtheria.
Δ = The diphtheria bacillus diagnosed on morphological grounds.
Δ = do. do. isolated and proved virulent.
H = The bacillus of Hofmann.
H = do. do. isolated and proved not virulent and incapable of producing acid.
O = No suspicious micro-organisms.
⊙ = No growth of any kind.

Name and reference number	Consecutive examinations					
Father 786				27.XII ⊙	31.XII O	8.I H
Mother				21.XII Δ	2.I O	8.I Δ
P. 17	24.X Δ	7.XI Δ	21.XI ⊙	21.XII Δ	2.I **H**	8.I Δ
Q. 74 D	24.X O	10.XI O	21.XI O	21.XII Δ	2.I **H**	8.I H
Sister 73	24.X O			21.XII O	2.I H	8.I H
Maid 779				21.XII Δ	2.I O	8.I O

The history just related affords also a good instance of the occasional long persistence of the virulent diphtheria bacilli in the pharynx. During the outbreak at Cambridge the bacilli as a rule were found to disappear very quickly from the throats of convalescents. In one man

however they persisted from October 31 to January 2nd. No attenuation of the bacilli has been observed in this case, nor in that of the boy *P.* Non-virulent diphtheria bacilli, as has already been mentioned, were but four times observed, once only in a convalescent and three times in a contacts who remained well.

The following Table shows some of the results of consecutive examinations and will serve to illustrate the necessity of more than one negative examination, and the persistency of the diphtheria bacillus in a number of cases.

TABLE II.

RESULTS OF SOME CONSECUTIVE EXAMINATIONS OF DIPHTHERIA PATIENTS AND OTHERS WHO WERE FOUND TO HARBOUR THE DIPHTHERIA BACILLUS.

Δ = Diphtheria bacillus proved by injection into animals. **H** = Hofmann's bacillus proved by injection into animals. O = No suspicious bacilli. Δ = Do. microscopic examination only. H = Do. microscopic examination only. ⊙ = No growth of any kind on the culture tube.

Initials and reference number		D = clinical case of diphtheria	Consecutive examinations								Remarks
G. N.	1	D	23.x Δ	7.xi O	13.xi O	15.xi **Δ**	20.xi O	24.xi O	27.xi O		Chron. membr. rhinitis
Gl. N.	58		24.x O	31.x O	3.xi Δ	19.xi O	10.xi H	14.xi O	16.xi O	19.xi O	
T. N.	59		24.x O	31.x Δ	3.xi H	10.xi O	15.xi O	16.xi O			
Mr N.	302	D		31.x O	3.x **Δ**	10.xi O	15.xi O	16.xi O			
Mrs N.	57	D	25.x **Δ**	31.x O	3.xi O	10.xi O	15.xi O	16.xi O			
A. G.	3	D	23.x Δ	6.xi O	7.xi O	16.xi O					
J. S.	9		24.x **Δ**	3.xi Δ	10.xi O	17.xi O	27.xi O				
Jk. S.	82		24.x O	3.xi **H**	10.xi O	17.xi H	27.xi **H**				
G. S.	83		24.x O	3.xi H	10.xi H	17.xi O	27.xi O				
D. S.	27		24.x O	3.xi H	10.xi H	17.xi O	27.xi H				
M. D. W.	45	D	24.x **H**	28.x H	7.xi O	12.xi O	14.xi H	16.xi O	19.xi O	22.xi O	24.xi O
M. C.	47	D	24.x Δ	7.xi O	13.xi **Δ**	15.xi O	17.xi O	20.xi O			
H. C.	115	D	26.x O	29.x **Δ**	31.x O	2.xi O	13.xi O	15.xi O	17.xi O	20.xi O	22.xi O | 24.xi O
M. H.	124		27.x H	7.xi O	12.xi O	14.xi O	16.xi O	24.xi O			
A. B.	172	D	28.x **H**	29.x H	7.xi H	12.xi O	14.xi O	16.xi O			
A. Mt.	260		30.x **Δ**[1]	3.xi O	8.xi **H**	12.xi H	14.xi O	16.xi O	19.xi O	24.xi O	[1] = non-pathogenic acid producing bacillus
S. Mt.	304		31.x H	8.xi **H**	12.xi H	14.xi O	16.xi O	19.xi H	22.xi O	24.xi O	27.xi H
W. N.	584		5.xi **H**	12.xi H	14.xi O	16.xi O	19.xi H	22.xi O	24.xi O	27.xi O	
Q. D.	283		30.x Δ **H**	7.xi H	12.xi O	14.xi O	16.xi O				
G. D.	244	D	29.x Δ	18.xi **H**	20.xi H						
Mrs M.	250		29.x **Δ**	12.xi Δ	24.xi O	27.xi H	30.xi **Δ**	18.xii O			
Mr J.	299		31.x **Δ**	18.xi Δ	20.xii Δ	2.i Δ					
G. D.	714	nose	5.xii H	11.xii **Δ**	15.xii O	20.xii H	21.xii O				Case of "scarlet fever"
		pharynx	5.xii H	11.xii O	15.xii O	20.xii H	21.xii O				
M. Cl.	723	D	1.xii **Δ**	17.xii Δ	20.xii **Δ**	24.xii **H**					
V. Th.	734	D	9.xii **Δ**	12.xii O	18.xii H	20.xii H	3.i O				
B. C.	792	D	28.xii **Δ**	2.i **Δ**	3.i Δ	7.i **Δ**(1) 7.i **H**(1)	9.i Δ	21.i Δ	28.i Δ 8.ii O	5.ii O 11.ii O	(1) On 7.i an almost pure culture of diphtheria bacilli from the throat and an almost pure culture of Hofmann from the nose
A.	735		10.xi O	12.xii O	13.xii O	14.xii O					Turned out to be scarlet fever

Table III.

Tests of Virulence of the Cultures Isolated.

(A) *Acid forming bacilli identical with one or other of the types of diphtheria bacillus.*

(a) *Virulent.*

List number and initials of person from whom culture was obtained	D = a notified case of diphtheria	Date of swab	Weight of guinea-pig	Quantity and age of broth culture injected		Condition of guinea-pig on the eleven days following injection. The letters refer to the local conditions about the seat of inoculation. T = trace of oedema, SS small swelling, MS medium swelling, LS large swelling, + death, N no local swelling											Remarks
						1	2	3	4	5	6	7	8	9	10	11	
			grms.	c.c.													
Mrs M. 250	D	29 . x	450	0·1	8 days	T	SS	LS	LS	LS	LS	LS	LS	LS	+		
,, 250a		30 . xi	240	0·1	2 ,,	T	SS	+									
Mr N. 302a	D	3 . xi	280	0·5	?	SS	+										
,, ,,		,,	180	0·05	2 days	T	+										
Mrs N. 57	D	25 . x	230	0·5	?	T	+										
G.N. 1a	D	15 . xi	390	0·1	2 days	SS	+										G.N. discovered on Oct. 23 to have a nasal discharge full of diphtheria bacilli. He had been going to school in this state for a fortnight
Son of the above		,,	260	0·025	,,	T	SS	LS	LS	LS	LS	LS	LS	LS	LS	+	
Mr J. 299	D	31 . x	260	0·5	?	T	+										
J.S. 9		24 . x	235	0·5	?	T	+										
C.W. 11		25 . x	230	0·5	?	+											
H.C. 115a	D	29 . x	350	0·1	4 days	SS	+										
J.C. 37		24 . x	500	0·1	7 days	SS	+										
R.S. 241	D	29 . x	200	0·1	3 days	SS	+										
R.W. 17		24 . x	250	0·1	15 days	SS	LS	LS	LS	LS	LS	+					
,, ,,		,,	300	0·1	2 ,,	SS	+										
,, 17a		21 . xii	280	0·1	3 ,,	SS	+										

M.C.	47a	D	13 . XI	215	0·1	2 days	T	SS	+								Instance of apparent increase of virulence of culture due to adaptation to laboratory media. 12 days elapsed between the inoculations
,,	,,		,,	200	0·05	,,	T	+									
M.Cl.	723	D	1 . XII	250	0·1	2 days	T	T	N	N	N	N	N	N			
,,	,,		,,	220	0·1	,,	SS	LS	+								do. do. 3 days' interval
,,	,,		,,	250	0·1	,,	SS	+									
,,	,,		,,	220	0·25	,,	SS	+									
,,	,,		,,	220	2·0 (1)	,,	SS	N	N	N	N	N	N				(1) With antitoxin
,,	723b		20 . XII	220	0·15	,,	SS	+									
V.Th.	784	D	18 . XII	250	0·1	2 days	SS	+									
G.D.	714a	D	11 . XII	250	0·1	2 days	SS	+									Culture during convalescence
,,	,,		,,	250	0·05	,,	SS	SS	SS	T	T	T	T				Small patch of necrosis
,,	,,		,,	270	2·0 (1)	,,	SS	N	N	N	N	N	N	N			(1) With antitoxin
R.G.		D	20 . XII	200	0·1	2 days		T	T	T	N	N	N				
,,			,,	200	0·1	,,	SS	+									
Mrs W.	778		21 . XII	220	0·1	2 days	SS	+									
P.	790	D	27 . XII	260	0·1	2 days	SS	+									
B.C.	792	D	28 . XII	220	0·1	1 day	T	+									
,,	792b		2 . I	180	0·1	2 days	MS	+									
,,	792c		7 . I	200	0·1	1 day	LS	+									
A.M.	788	D	27 . XII	270	0·1	2 days	SS	+									

(*b*) *Not virulent.*

A.Mt.	260		30 . X	430	0·1	2 days	T		N	N	N	N	N	N	N	N	
,,	,,		,,	250	1·0	,,	SS	SS	T	N	N	N					
,,	,,		,,	180	2·0	,,	SS	T	T	T	T						
A.G.	721	D	30 . XI	265	0·1	3 days	SS	N	N	N	N	N	N	N	N	N	Culture during convalescence
,,	,,		,,	210	2·0	2 days	SS	T	T	T	N	N	N				
,,	,,		,,	240	2·0	,,	SS	T	N	N	N	N	N				
B.	782	D	24 . XII	185	0·1	2 days	T	N	N	N	N	N	N				
,,	,,		,,	220	0·1	,,	T	N	N	N	N						
,,	,,		,,	200	2·0	,,	T	N	N	N	N	N	N	N	N		
Mrs C.	793		28 . XII	290	0·1	2 days	T	N	N	N	N	N	N	N	N	N	
,,	,,		,,	230	2·0	,,	N	N	N	N	N	N	N	N			

TABLE III. (*cont.*)

(B) *Short bacilli of the Löffler-Hofmann type which do not produce acid in glucose broth.*

List number and initials of person from whom culture was obtained		D = a clin. case of diphtheria	Date of swab	Wt. of guinea-pig	Quantity and age of culture injected		Result: {N = No local swelling. SS = Small swelling. T = Trace.}										Remarks
				grms.	c.c.		1	2	3	4	5	6	7	8	9	10	11	
G.G.	375		1 . XI	370	0·5	?	T	N	N	N	N	N	N	N	N			
,,	,,		,,	430	0·5	3 days	SS	N	N	N	N	N	N	N	N	N	N	
,,	,,		,,	450	2·0	3 ,,	SS	T	N	N	N	N	N	N	N	N	N	
,,	,,		,,	230	2·0	48 hrs	SS	T	N	N	N	N	N					
F.T.	307		31 . X	350	2·0	?	T	T	N	N	N	N	N	N				
			,,	200	2·0	48 hrs	T	N	N	N	N	N	N					
Q.D.	283		30 . X	560	2·0	48 hrs	SS	N	N	N	N	N	N	N	N	N		
,,	,,		,,	190	2·0	3 days	T	N	N	N	N	N						
W.R.	269		30 . X	230	2·0	7 days	T	N	N	N	N	N	N	N	N	N		
,,	,,		,,	230	2·0	48 hrs	SS	N	N	N	N							
A.B.	172	D	27 . X	420	0·1		T	N	N	N	N	N	N	N	N	N		
,,	172a		1 . XI	500	2·0	3 days	SS	N	N	N	N	N	N	N	+[1]			
,,	,,		,,	406	2·0	7 ,,	T	N	N	N	N	N	N	N				Remained well
L.M.	422		1 . XI	400	0·1		N	N	N	N	N	N	N	N	N	N		
E.U.	432		2 . XI	430	0·1		T	N	N	N	N	N	N	N	N	N		
,,	,,		,,	230	2·0	6 days	T	N	N	N	N	N	N	N				
P.H.	434		2 . XI	400	2·0		T	T	T	N	N	N	N	N	N			
L.P.	570		6 . XI	520	2·0	3 days	T	T	N	N	N	N	N	N				
C.G.	491		3 . XI	480	0·5		SS	T	N	N	N	N	N	N	N	+[1]		
,,	,,		,,	240	2·0		T	T	N	N	N	N	N					Remained well
L.N.	475		3 . XI	310	2·0		SS	T	N	N	N	N	N					

F.T.	265		30 . x	220	2·0	6 days	N	N	+[1]	N	N	N	N	N	N		
,,	,,		,,	250	1·0	3 ,,	T	N	N								
B.K.	315		31 . x	220	2·0	6 days	T	N	N	N	N	N	N	N	+[1]		Remained well
,,	,,		,,	200	1·5	3 ,,	T	N	N	N	N	N					
A.S.	411		1 . XI	220	2·0	6 days	T	N	N	N	N	N	N	N			
L.A.	545		3 . XI	230	2·0	6 days	T	N	N	N	N	N	N	+[1]			Remained well
,,	,,		,,	200	1·5	3 ,,	T	N	N	N	N	N					
W.A.	546		3 . XI	230	1·5	3 days	T	N	N	N	N	N					
F.S.	409		1 . XI	220	2·0	3 days	T	N	N	N	N	N					
J.B.	499		3 . XI	200	1·0	3 days	T	N	N	N	N	N	N	N			
P.	460		3 . XI	430	2·0	3 days		N	N	N	N	N	N	N	N		
,,	,,		,,	630	2·0	3 ,,		N	N	N	N	N	N	N	N		
A.Bs.	502		3 . XI	430	2·0	11 days		T	N	N	N	N	N	N	N		
M.Ch.	398		1 . XI	350	2·0	3 days		N	N	N	N	N	N	N	N		
L.D.	439		2 . XI	310	2·0	10 days		N	N	N	N	N	N	N	N		
,,	,,		,,	310	1·5	2 ,,	SS	T	T	N	N	N	N				
W.N.	584		5 . XI	340	2·0	?		T	N	N	N	N	N	N	N		
,,	,,		,,	330	2·0	2 days	T	T	N	N	N	N	N				
E.G.	495		3 . XI	270	2·0	11 days		T	N	N	N	N	N	N	N		
D.G.	496		3 . XI	260	2·0	4 days	SS	N	N	N	N	N	N	N	N		
E.G.	80		24 . X	270	2·0	?	T	T	N	N	N	N	N	N	N		
M.D.W.	45	D	24 . X	240	2·0	4 days	T	T	N	N	N	N	N	N	N		
B.B.	451		2 . XI	220	2·0	4 days	SS	T	N	N	N	N	N	N	N		
A.B.	498		3 . XI	450	2·0	16 days	SS	T	N	N	N	N	N	N			
A.W.	542		3 . XI	430	2·0	7 days	SS	T	N	N	N	N	N	N			

[1] Autopsy: no local œdema; no pleural exudation. These deaths all happened in one week. The injections were repeated and the animals remained healthy. Death was thus evidently not due to the bacillus in question.

TABLE III. (*cont.*)

List number and initials of person from whom culture was obtained	D = a clinical case of diphtheria	Date of swab	Weight of guinea-pig grms.	Quantity and age of culture injected c.c.		Result: N = No local swelling. SS = Small swelling. T = Trace. 1	2	3	4	5	6	7	8	9	10	11	Remarks
F.W. 541		3 . XI	340	2·0	7 days	SS	T	N	N	N	N	N	N				
C.S. 395		1 . XI	500	2·0	7 days	SS	N	N	N	N	N	N	N				
H.H. 443		2 . XI	370	2·0	7 days	T	T	N	N	N	N	N	N				
E.M. 558		6 . XI	190	1·0	4 days	N	N	N	N	N	N						
A.Mn. 293	D	30 . X	200	1·0	4 days	N	N	N	N	N	N	N	N	N			
S.Mt. 304a		8 . XI	200	1·0	8 days	T	N	N	N	N	N	N	N				
A.Mt. 260b		8 . XI	200	1·0	2 days	N	N	N	N	N	N	N	N	N			
E.R. 517		3 . XI	210	1·0	2 days	T	N	N	N	N	N	N	N	N			
P.T. 404		1 . XI	200	2·0	12 days	T	N	N	N	N	N	N	N	N			
H.M. 162a	D	27 . X	230	1·0	2 days	T	N	N	N	N	N	N	N				
M.W. 84		24 . X	210	1·0	2 days	T	N	N	N	N	N	N	N				
R. 560		6 . XI	240	1·5	3 days	T	N	N	N	N	N	N					
F.B. 344		31 . X	225	1·5	3 days	T	T	N	N	N	N	N					
A.H. 505		3 . XI	240	1·5	3 days	T	N	N	N	N	N	N					
M.M. 538		3 . XI	420	1·5	3 days	SS	T	N	N	N	N	N					
N.H. 251		29 . X	230	1·5	2 days	T	T	N	N	N	N	N					
S.Cl. 511		3 . XI	370	1·5	2 days	SS	T	T	N	N	N	N					
B.Cl. 514		3 . XI	370	2·0	2 days	T	N	N	N	N	N	N					

E.Cl. 512		3 . XI	470	2·0	2 days	SS	T	T	N	N							
E.N. 391		1 . XI	260	2·0	2 days	T	T	N	N	N	N						
G.T.R. 25		17 . XI	220	2·0	2 days	T	N	N	N	N	N						
M.E.R. 26		17 . XI	240	1·5	?	SS	T	T	N	N	N	N	N				
C.M. 672		14 . XI	180	1·0	2 days	T	N	N	N	N	N	N	N	N			
G.H. 445		2 . XI	180	1·0	2 days	N	N	N	N	N	N	N	N	N			
A.C.R. 28		17 . XI	220	0·5	2 days	T	T	N	N	N	N	N	N	N			
Jk.S. 82a		24 . X	180	1·5	2 days	T	T	T	T	N	N	N	N				
,, 82d		10 . XI	230	1·0	2 ,,	N	N	N	N	N	N	N	N				
G.D. 324		31 . X	180	1·5	2 days	T	N	N	N	N	N	N	N				
J.B. 499		3 . XI	200	2·0	4 days	T	N	N	N	N	N						
,, ,,		,,	200	2·0	4 ,,	SS	T	N	N	N	N						
D.Cl. 733		8 . XII	250	2·0	2 days	SS	N	N	N	N							
G.D. 244a	D	20 . XII	210	2·0	2 days		T	N	N	N	N	N				Culture during convalescence	
A.L. 769	D	20 . XII	220	2·0	2 days		T	N	N	N	N					Culture during convalescence	
W.R. 799		28 . XII	200	2·0	2 days	SS	T	N	N	N	N	N					
L.C. 798		28 . XII	205	2·0	1 day	SS	T	N	N	N	N	N					
A.C. 806		31 . XI	250	2·0	2 days	SS	N	N	N	N	N	N	N				
M.C. 723c	D	24 . XII	250	2·0	2 days	SS	T	N	N	N	N	N	N			Culture during convalescence	
R.A. 796a		28 . XII	180	2·0	2 days	T	N	N	N	N	N	N	N				
R.W. 17e		21 . XII	200	2·0	2 days	N	N	N	N	N	N	N	N				

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Table III. gives a complete list of the guinea-pig inoculations which were made with the isolated cultures. Except in a few instances an autopsy was made on the animals which died, and all those which had been injected with *acid producing bacilli* were found to have the characteristic signs which follow the injection of diphtheria bacilli or their toxins into the subcutaneous tissue of guinea-pigs.

Conclusions.

1. Experience of the outbreak in Cambridge gave no reason for thinking that the pseudo-diphtheria bacillus is other than perfectly innocuous to man.

2. The relationship between the pseudo-diphtheria and the diphtheria bacillus remains undecided. Even though it should be definitely established that by laboratory procedures the former can be converted into the latter, it must yet be shown that the change occurs under natural conditions.

3. The frequent presence of the pseudo-diphtheria bacillus should not be allowed to weaken our efforts to detect and isolate those who harbour the virulent bacillus.

4. The principal means of combating diphtheria are, after the isolation of persons actually sick, the detection of those who go about apparently in good health carrying with them the diphtheria bacillus, and the isolation of such persons, and of convalescents from the disease until diphtheria bacilli can no longer be cultivated from them. No doubt the satisfactory isolation of healthy persons who carry about the bacillus will often prove impracticable. In such cases the infectious persons should be warned that they are a danger to others, and instructed to take certain precautions, which need not be detailed here. In the case of children isolation will usually be practical, and experience among the poorer classes at Cambridge has shown that parents can usually be brought to consent to the removal of their children to an isolation home.

5. With increasing confidence in the bacteriological test on the part of the medical profession and of the general public, such measures will be much facilitated. But this confidence will not be forthcoming until bacteriological examination distinguishes clearly between the diphtheria and the pseudo-diphtheria bacillus.

6. The prophylactic use of antitoxin during the outbreak of diphtheria at Cambridge proved of great value. It ought always to be accompanied by bacteriological examination, lest by the suppression of symptoms of disease it be the means of preventing the isolation of those who would have fallen ill, and of allowing them to go free and distribute infection. The need of this precaution is strengthened by the fact that the few persons who continued to harbour the diphtheria bacillus for a long time were among those who in consequence of a timely dose of antitoxin, or because they were naturally insusceptible, were little or not at all affected. It seems probable that an active inflammatory reaction is instrumental in expelling the bacilli, which are consequently more apt to persist if this is prevented.

EXPLANATION OF PLATES.

The initials and numbers placed below the drawings correspond to those in the tables, so that various particulars about the culture represented can be ascertained.

In one case no number is given, the preparation being from a case not included in the tables.

Except when stated otherwise the drawings were made from the original serum culture after 24 hours' incubation.

When drawings of more than one culture taken at different times from the same person are given the date of each is given for reference to Table II.

The drawings ($\times$ 1000) were made with a Camera Lucida, a Zeiss oil immersion $\frac{1}{12}$ in. Objective, and Eyepiece C.

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