THE DISTRIBUTION OF THE DIPHTHERIA BACILLUS AND THE BACILLUS OF HOFMANN IN THE THROATS OF "CONTACTS" AND NORMAL PERSONS.

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Attempts have been now made in various towns, schools, and institutions to stamp out epidemics of diphtheria by isolating the sick until their throats have been proved by bacteriological examination to be free from the diphtheria bacilli.

In several of these cases, acting on the well-known fact that persons who have come in contact with the diseased may acquire and carry in their throats and noses virulent diphtheria bacilli without themselves being ill, "contacts" have been examined, and those found to be harbouring diphtheria bacilli isolated in the same manner as the convalescents.

These efforts to check the spread of the disease have for the most part been based on the assumption that virulent diphtheria bacilli do not occur in the mouths and noses of persons who have not been in some way exposed to persons suffering from the disease, or persons who have acquired the bacillus by being so exposed. This view is not however held by all the authorities on the subject; for while many are of the opinion that virulent diphtheria bacilli are never to be found in the throats of healthy persons, who have had no opportunity of acquiring the bacillus by contact, others believe that it does occur in small numbers amongst the normal population.

Should the view of the latter school be accepted the method of attempting to suppress epidemics by the isolation of healthy individuals is not only likely to prove useless, but entails unnecessary hardships on the isolated persons.

It is with the purpose of showing that virulent diphtheria bacilli do not exist in the noses and throats of healthy persons, who have had no opportunity of acquiring them by contact, and that the method of isolating convalescents and healthy persons harbouring the bacillus is consequently likely to be of great benefit in suppressing epidemics of the disease that the following statistics have been brought together.

THE VIEWS OF VARIOUS AUTHORITIES, AND THE STATISTICS ON WHICH THEY ARE BASED.

The Occurrence of Diphtheria Bacilli in notified cases.

Novy[71] gives a table showing the results of observations by European workers between 1886 and 1895, in which 2,846 cases of diphtheria were examined and diphtheria bacilli found 2,344 times (82·4 %).

European and American observers combined, examined 8,186 cases, finding the specific bacillus in 5,943 or 72·6 per cent.

French investigators in the Institut Pasteur obtained the bacillus in 701 out of 960 cases (73 %).

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[79] showed that out of 5,340 suspected cases, 67·5 % were true diphtheria.

Woodhead[101] 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.

Cobbett[18, 20] in two outbreaks at Cambridge, in 1900 and 1901, found diphtheria bacilli in 57 % (42 cases), and 66·6 % (27 cases) of notified cases. Except in one of the negative cases two or more subsequent examinations made by him confirmed the original diagnosis.

At Colchester[41] diphtheria bacilli were found in 87 % of notified cases. Repeated examinations were made of the negative cases.

The results of nearly 27,000 certified cases quoted above show that there was bacteriological evidence of diphtheria in about 72 %.

The Occurrence of Diphtheria Bacilli in persons who have been in contact with cases of the disease, or with others who acquired the bacillus in this way.

All observers are agreed that virulent and dangerous diphtheria bacilli occur in the mouths of certain healthy persons who have come in contact with the sick, or with others who, like themselves, harbour diphtheria bacilli.

The proportion of infected to non-infected contacts 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 finds.

Families. Cobbett[18] found every member of one family to have diphtheria bacilli in their throats. From three the organisms were isolated and found to be virulent (100 %).

Spirig[88] examined the children of two families numbering four and six respectively. There was one case of clinical diphtheria—of the remaining nine children six were found to harbour diphtheria bacilli, one Hofmann's bacillus, and two no bacilli; out of the infected six five subsequently developed diphtheria. Infected contacts 66·6 per cent.

Park and Beebe[78] amongst 48 children from 14 infected families found bacilli in 50%. Of six cultures tested all were virulent.

Schools. Goadby[38] examined a school with 600 children—21 cases of diphtheria had previously occurred. He found diphtheria bacilli present in 190 cases (34·1 %). The morphology in culture was alone relied on.

Berry and Washbourn[8] out of 142 girls examined in a school, in which several cases of diphtheria had occurred, discovered diphtheria bacilli in 17 (11·9 %).

Amongst 200 scholars in a truant-school Denny[29] found 22 with diphtheria bacilli shortly after four cases of true diphtheria had occurred (11 %).

Hospital Wards. Chatin and Lesieur[17] 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; the remaining 61 were free—(2·66 %).

Park and Beebe[78] found 6 out of 55 children in a foundling hospital

to harbour diphtheria bacilli. Of these 5 were virulent. Some cases of diphtheria had from time to time occurred in this institution (virulent bacilli 9 %).

Lister [61] in the Shadwell Hospital examined 125 children, 69 of whom had nasal discharges, and found 61 (24 with discharges) had diphtheria bacilli (48 %).

Müller [70] made observations on 100 children in a general ward in the Charité in Berlin. Six had diphtheria bacilli without illness on admission. Two days later bacilli were found in 14 others (20 %).

General Contacts. Kober [56] at Breslau showed that 15 out of 128 general contacts harboured diphtheria bacilli (11·7 %).

Meade Bolton [67] stated that of 214 healthy persons examined who had previously been exposed to infection 45·5 % showed diphtheria bacilli.

Cobbett [19] during an outbreak at Cambridge in 1900, on examining 650 persons, mostly school-children, found 19 (2·9 %) affected with diphtheria bacilli. Experiments on animals showed that out of nine pure cultures tested, three were not pathogenic to guinea-pigs.

In the Colchester [41] epidemic I found 54 persons (10·4 %) harboured diphtheria bacilli out of 519 examined. All these were school-children or persons connected with schools. Morphological and cultural methods were relied on as no tests for pathogenicity could be undertaken there.

Contacts with Sore Throats.

Goadby [38] noted in his cases that out of the 586 examined 262 had enlarged tonsils, and 196 abnormal throats. 34·1 % harboured diphtheria bacilli. Pakes [74] examined 3,000 cases of sore throat, finding diphtheria bacilli in 343 (14·3 %).

The mean of the examinations of the very close contacts in families gives 51 % of infected persons; in more distant contacts in institutions 40 %, and in schools 24 %; whilst in the class of general contacts only 12 % harbour diphtheria bacilli.

The results of the examination of healthy persons who have not recently been in contact with the disease.

Kober [56] examined 600 healthy school-children and discovered diphtheria bacilli in 15 cases. The bacilli from 10 were however found to be non-pathogenic. Of the five children with virulent bacilli one

sat at school next a child who had diphtheria eight weeks before, three were playmates of neighbours' children who had had diphtheria recently, and the fifth had associated with a family in which a fatal case of the same disease had occurred 10 weeks before. Five of those with non-virulent bacilli were proved also to be old contacts (non-contacts with virulent diphtheria bacilli 0 %).

Garratt and Washbourn [37] examined 666 cases of scarlet-fever admitted under their care at the London Fever Hospital, and found in 8 (1·2 %) cases bacilli morphologically identical with the diphtheria bacillus; their examinations were conducted on a class of persons especially prone to acquire the diphtheria bacillus if brought in contact with the disease. The virulence of the organisms was not tested.

Denny [29] of Brooklyn, Mass., examined 235 healthy individuals (216 children and 19 adults), a large proportion of the well-to-do class. 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. The bacilli were so few that a pure culture could not be obtained (·4 %).

Park and Beebe [77] examined 330 persons, chiefly hospital patients. Diphtheria bacilli were obtained in culture from 32 persons, but 24 cultures proved to be non-virulent. The presence of all but two of the eight virulent examples was accounted for by recent contact, and these two occurred in adults. (Virulent bacilli unaccounted for by contact in ·6 % of these persons.)

Goadby [38] examined 100 school-children from a school in which there had been no diphtheria for two years and found 18 with diphtheria bacilli. This school was examined as a control to his previous experiment in which diphtheria was present (see p. 218). The disease was therefore prevalent in the neighbourhood and the high percentage of diphtheria (18 %) is to some extent accounted for. Morphology was alone relied on, and virulence was not tested.

Herman Biggs [9] examined 330 healthy persons and found virulent diphtheria bacilli in eight, and non-virulent acid-producing bacilli in 24 (2·4 % of virulent bacilli).

The Committee [65] of the Massachusetts Association of Boards of Health feel justified in the inference that in urban communities at least 1 to 2 % of well persons amongst the general public are infected with diphtheria bacilli; but their experiments show that only 17 % of these bacilli are virulent. In other words that 17 out of 5,000 to 10,000 of all persons harbour diphtheria bacilli which are dangerous to public health.

Their figures[1] show however that in the following places, Ontario, Newton, Springfield, Washington, Lowell, Waltham, and Providence, in which 50, 63, 185, 221, 250, 297, and 927 persons respectively were examined, the typical bacilli were only found as follows, 0, 0, 0, 2, 2, 2, 4, namely 10 cultures with typical bacilli in 1993 persons (·5 %). If only 17 % of these are virulent the percentage of virulent bacilli in the places mentioned is only ·085.

In New York, Brooklyn, and Minnesota on the other hand, where it is stated that diphtheria was prevalent at the time of these investigations, 3·66 %, 2·32 %, and 2·89 % of persons harbouring diphtheria bacilli were found amongst 82,129 and 4,250 persons respectively; whether or not diphtheria was present in Boston is not mentioned, but in this city 27 infected persons were found out of 892 examined, giving a percentage of 3·02.

Pugh [81], working in the North-Eastern Fever Hospital, has come to the conclusion 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."

Hewlett and Murray [46] investigated the throats of all children (385) admitted into the Victoria Hospital. (The cultures were examined at the Jenner Institute of Preventive Medicine.) They found diphtheria bacilli in 58, or 15 %—only three cultures were examined for virulence and of these two were stated to be only slightly, if at all, virulent.

Except in these three morphology was alone relied on.

As a result of their examinations Hewlett and Murray argue that 15 %, or one out of every seven, of normal children amongst the general community have diphtheria bacilli in their throats. They say "the morals deducible from these figures are almost too obvious to need detailed statement: it is clear that babies and young children are

[1] In their table the authors of this report include Boston, New York, and Brooklyn, with the places quoted in one set. I have here separated the three places just mentioned for reasons given in the text.

not the innocent and harmless creatures usually imagined, and that kissing and other similar signs of artificial demonstration of childish affection should be discouraged."

Their results are entirely at variance with the other observations which have been quoted.

Although these authors do not as far as I am aware draw from their experiences the inference that the isolation of infected contacts can be of little importance, scarcely any other construction can be placed upon their observations. Moreover they seem to have made no inquiries into the antecedents of their patients and do not state whether diphtheria was prevalent, or not, at the time.

The following table shows the prevalence of diphtheria bacilli amongst normal persons as ascertained by investigations in which no inquiries as to contact appear to have been instituted.

Observer	Persons examined	Organisms morphologically resembling diphtheria bacilli	Virulent diphtheria bacilli	Non-virulent diphtheria bacilli
Massachusetts Committee [65]	1993	10	—	—
Garratt and Washbourn [37]	666	8	—	—
Hewlett and Murray [46]	385	58	1?	2
Herman Biggs [9]	330	32	8	24
	3374	108 (3·2 %)	9 (·2 %)	26 (·7 %)

It is very noticeable in such statistics that whenever the virulence of the organisms discovered has been tested a large proportion have been found to be non-pathogenic. In the above list 26 out of the 35 (74 %) tested turned out to be devoid of virulence.

The following table, on the other hand, shows the results of observations on normal throats in which careful inquiries were instituted as to the possibility of recent infection.

Observer	Persons examined	Virulent diphtheria bacilli	Non-virulent diphtheria bacilli	
Kober [56] [1]	590	0	5	
Park and Beebe [77]	324	2	24	
Denny [29]	235	1	0	Virulence not tested
	1149	3 (·26 %)	29 (2·5 %)	

The various figures which have been quoted merely emphasise the well-known facts that diphtheria bacilli are present in the large majority of cases which have been diagnosed as diphtheria on clinical

[1] Persons found on inquiry to be "contacts" have been excluded in this table.

grounds alone, and that contacts with clinical cases, as well as persons who have been associated only with the latter, are liable to become infected with diphtheria bacilli. The proportion of infected persons depends, as has been shown, on their relationship to the clinical cases.

On the other hand, amongst persons who have had no opportunity, as far as can be ascertained, of acquiring the bacilli from clinical cases, or "contacts," virulent bacilli are present in very small numbers.

Some investigators have apparently relied entirely on the morphological characters of the bacilli, but others have not only cultivated them but have tested their pathogenic power. The results of the latter are naturally the most trustworthy. They have shown that in a large proportion of their cases in which bacilli morphologically and culturally identical with diphtheria bacilli have been found they were devoid of virulence. On further inquiry amongst those who harboured virulent bacilli they have elicited the fact that in reality they were, in almost every case, recent contacts. After eliminating all such recent contacts it is found that virulent diphtheria bacilli occurred, and could not be satisfactorily accounted for, in two out of 1149 persons. In a third person bacilli morphologically identical with diphtheria bacilli were discovered, but were too few in number to allow of a pure culture being obtained.

I think it may therefore be safely assumed in the absence of conclusive evidence to the contrary that virulent diphtheria bacilli are seldom if ever present in the throats of healthy persons, who have not been in contact with cases of diphtheria, or infected contacts.

The Results of Isolation.

The attempts which have been made at Cambridge[18–20] and Colchester[41] to stamp out diphtheria epidemics by isolation have been attended by encouraging results. The measures employed depended on the assumption that virulent diphtheria bacilli do not occur in the mouths of healthy persons (non-contacts). The methods by which suitable persons were examined and isolated are given at length in the papers mentioned, but the main points may be recapitulated here.

(1) As far as possible all notified cases were examined, and isolated until three consecutive negative examinations showed them to be free from diphtheria bacilli.

(2) As far as possible all cases of sore throat brought to the notice

of the medical practitioners, and more especially those occurring amongst school-children, were investigated.

(3) As far as possible all children belonging to the families in which diphtheria cases had occurred, and all persons known, or likely, to have been in contact with them, were also examined, and if diphtheria bacilli were found in their throats, isolated. Particular attention was paid to school contacts.

(4) The means by which the disease is generally considered to be communicated to others by patients and contacts were explained to the school teachers, and precautions taken to guard against its spread by infected articles.

(5) The administration of antitoxin as a prophylactic to healthy contacts, who showed the bacillus in their throats, was encouraged, as was also the use of antiseptic mouth-washes.

At Cambridge [18] in 1900 the results of these measures were highly satisfactory, and their application in the following spring [20] was again successful.

At Colchester [41] the most striking results were obtained in checking the progress of the disease in the schools. These are given in tabular form in the paper referred to, but the general results may I think be again stated here with advantage. Cases of diphtheria were of constant occurrence amongst the scholars until all known healthy contacts had been carefully examined and those harbouring diphtheria bacilli excluded from school. On the reopening of the schools after the completion of the precautionary measures no case of diphtheria was notified amongst the scholars except in three out of 19 schools.

In the first of these three schools eight weeks elapsed, then three cases were notified. (One died before any cultivation could be made, and in the other two no bacilli could be found.) In the second school two cases occurred after a lapse of four weeks; but in the third there was a small outbreak extending over four weeks. All the scholars (112) in the infected portion of this school were examined, and five harbouring diphtheria bacilli were found, and excluded. Subsequently no further cases were notified[1].

In this outbreak the administration of antitoxin without bacteriological examination was not encouraged, as persons thus

[1] The cases mentioned are the only ones which occurred amongst the school-children during the 10 weeks which followed the opening of the schools. After that period I was no longer in a position to carry on the record.

rendered immune carry the organisms in their mouth for long periods and may act as unknown centres of infection[1].

Berry [8] gives the results of isolating infected children in a girls' school with 200 scholars. Diphtheria was introduced (Feb. 25th) after the Christmas holidays. A series of mild sore throats followed till March 27th, when another case of the disease occurred, together with three examples of sore throat associated with the diphtheria bacillus. These persons were isolated. A further series of sore throats however continued till April 30th, when five cases of diphtheria were notified. Washbourn then examined all scholars with any abnormality of the throat (142), and discovered diphtheria bacilli in 17 of them. All the latter were isolated and not allowed to return to school till they had been declared free. No further cases of diphtheria, or sore throat, occurred.

A good example of the advantages of efficient isolation is given by Burnett [14], and Peck [80] cites the case of a school in which the effect of isolation was for a time very beneficial. An abstract of these cases is given later (p. 239).

Goadby [38] found some benefit resulted from partial isolation in a school, although the system eventually broke down.

In Providence [65] the method of keeping at home the infected members of families in which diphtheria exists was carried out faithfully for a period of five years, but has been abandoned because it met with a very decided opposition from both the laity and medical profession. It should be noted that in Providence only one negative culture-test was required for release.

Wesbrook, Wilson, and McDaniel [97] attempted to stamp out diphtheria in a school by the isolation of the infected children. Although their method of isolation eventually broke down they assert that "the effects of the isolation, and the thorough looking over to which the children were subjected, as well as the local treatment of the throat and nose in ridding them of diphtheria bacilli had an apparent beneficial effect on the general health of the school."

The examples which have just been cited clearly demonstrate the advantages to be derived from thorough examination, and isolation, whenever practicable. Even in those cases in which isolation was not

[1] Instances of persons thus temporarily rendered immune acquiring the disease without further contact are given by Jump [52], and experimental observations on this subject have been made by Bullock [13].

efficiently carried out the observers considered that some good effects followed.

Cobbett[21] considers that "the duty of discovering, isolating, and disinfecting the former class of persons (infected with virulent bacilli) is becoming more and more the urgent duty of the sanitary authorities. For the fact that they are not scattered broadcast throughout the community as was once supposed but are confined to the class of persons whom we conveniently term '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."

By no means all observers are however in complete agreement with this opinion. Several investigators, while acknowledging the danger of infection to others through healthy infected contacts, at the same time do not agree as to the practicability of their isolation.

The Massachusetts Committee[65] arrived at the following conclusions. "There are scattered among the general public a considerable number of persons, not recently and directly exposed to the disease of diphtheria, who have typical diphtheria bacilli in their throats." They compute that there would be 8000 such in Boston, and continue, "the mere statement of this fact shows how entirely futile it is to attempt to seek out and isolate the whole of this number. If this cannot be done it is useless and unjust to isolate the small number that it may be possible to discover."

They advise however that children in infected families should be kept away from school and public places; that teachers, nurses, and others who are brought in close contact with children, as well as milk-men, should not be allowed to continue their work if found to be harbouring diphtheria bacilli.

They also express the opinion that in schools and institutions, if the infection is not too wide-spread, infected persons, whether sick or well, should be isolated until free from bacilli.

Further, they consider that when diphtheria appears in a community which has been for some time free from it, it is advisable to isolate all persons who have been brought in contact with the patient until it should have been shown that they are free from diphtheria bacilli. Finally, they advise that the dangers of infection should be pointed out to the infected individuals, or their guardians, as well as to school

teachers, and means indicated for minimising the danger of infection by articles used by patients or contacts[1].

Prof. Wesbrook, one of the signatories to the report, modifies his assent as follows. "It may sometimes be impracticable to isolate from the public all the well persons in infected families, schools or institutions, though it should be done as a routine if at all possible."

Welch[96] in summarizing the results of work on this subject up to 1894 remarks, "all members of an infected household should be regarded as under suspicion, and, where isolation is not enforced, the healthy as well as the sick should be prevented from mingling with others until cultures, or a sufficient lapse of time, give the presumption that they are not carriers of infection."

From these quotations it is seen that while some authorities consider that all infected persons harbouring virulent bacilli should be isolated, others think that this should only be done under special circumstances, and others again would merely isolate the infected members of families in which diphtheria exists.

On the other hand, it would certainly appear from the deductions of certain bacteriologists as to the frequency of diphtheria bacilli in normal throats, that they regard isolation as of little value.

If it be admitted then in accordance with the great bulk of expert opinion, that the isolation of infected persons is a necessary measure for checking outbreaks in schools, institutions, and towns, the following questions have to be considered in detail by the authorities. Which of the various types of bacilli should be considered dangerous? How many consecutive negative examinations are necessary before infected persons, whether convalescents or healthy contacts, can be released from isolation? How long are diphtheria bacilli likely to persist in the throats of these persons? How are the bacilli communicated by infected persons to others? What classes of persons should be examined, and, if found infected, isolated? Can infection be carried otherwise than through infected persons?

Which of the various types of bacilli should be considered dangerous?

The divergent views held on this question even at the present day are well illustrated in the Report of the Massachusetts Committee[65].

[1] This report is signed by C. V. Chapin, H. W. Hill, S. W. Abbott, F. H. Baker, F. P. Denny, E. P. Gorham, W. H. Gore, A. Hudson, T. B. Shea, Theobald Smith, and F. F. Wesbrook.

The several collaborators were requested to detail the various bacilli they found according to Wesbrook's [98] types, and also to state in each case whether or not a positive diagnosis of the presence of diphtheria bacilli had been made.

In Providence, 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. If all granular or barred forms, but not solid forms, be included, as Prof. Gorham of Providence states, there would be 3 % of positives. If all be included there would be about 25 %. The number actually reported positive makes about 9 %.

In Washington the positives formed ·9 % on the Committee's standard; but 22 % were actually reported positive. In Boston on the Committee's standard 3·02 % were positive; but only 1 % was so reported.

It is evident from the above that some standard must be adopted in dealing with an outbreak.

Cobbett [19] has carefully worked out the virulence of the various types of bacilli met with during the epidemic of 1900 at Cambridge. A standard based on these observations was adopted by him in the outbreaks of 1900 and 1901 at Cambridge, and by myself at Colchester [41].

He recognises five morphological types of diphtheria bacilli from young serum cultures[1]:

(1) Oval bacilli with one unstained septum, very young forms[2].
(2) Long, faintly stained, irregularly beaded bacilli.
(3) Regularly beaded bacilli, streptococcal forms.
(4) Segmented bacilli.
(5) Uniformly stained bacilli.

The majority showed polar bodies by Neisser's method of staining in cultures less than 24 hours old, grown at 37° C., and all formed acid in 48 hours when grown in glucose broth (1 %) in pure culture.

Cultures grown in broth for 48 hours were injected subcutaneously into guinea-pigs of 200—500 g. in doses of ·1 c.c. In such doses 25 virulent bacilli killed within three days. In four examples of non-virulent diphtheria bacilli doses of 2 c.c. did not cause death. Cobbett found no intermediate degrees of virulence amongst diphtheria bacilli.

[1] The medium used for growing the cultures was alkaline ox serum, to which 1 % of glucose had been added. First proposed by Prof. Lorrain Smith, of Belfast. *Brit. Med. Journ.* Vol. II. 1894.

[2] The author does not mean to imply that in any pure cultures these would at any time be the only forms met with. Certainly in my experience this has never been the case.

At Colchester [41] many pure cultures were isolated in the following way. The top of a suspicious colony was touched with the point of a sterile platinum needle. The needle was then transferred to a tube of sterile salt solution (·7 %) and, after its removal from the fluid, a coverslip preparation was immediately made with it. If the latter showed by microscopic examination the desired organisms and no others, a serum subculture was sown from the salt solution tube. In some cases a second subculture from the first was necessary before a pure culture could be obtained.

All organisms which were considered on morphological grounds alone to be diphtheria bacilli, and which were isolated and tested, were found to form acid in glucose broth. Nearly all stained well by Neisser's method and in young subcultures retained the appearance of diphtheria bacilli. At the time there was no means of carrying the investigation further and testing their pathogenic properties.

Almost every original cultivation, in which organisms resembling diphtheria or Hofmann's bacilli occurred, was stained by Neisser's method, or Cobbett's modification [21] of it, and, except in very few instances, it was found that diphtheria bacilli showed polar bodies, whereas Hofmann's bacilli did not—about 2000 cultures were examined.

Cobbett [19] observes that "the bacillus of Hofmann in young serum cultures appears with considerable regularity as a darkly staining oval bacillus of somewhat variable length, with one 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 then the diagnosis is more difficult." He found that they showed no polar bodies by Neisser's method. Of 69 pure cultures of this organism which he isolated, and tested, not one produced acid in glucose broth or caused any local oedema in guinea-pigs. He finally says that "once one had become well acquainted with the range in its variation it was fairly easy to recognise the diphtheria bacillus and distinguish it from all others"; but goes on to remark "that the eye cannot become 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." No short Hofmann-like virulent bacilli such as have been described by Wesbrook were encountered by him.

I have, however, met with colonies of segmented bacilli in which

very few, if any, of the small typical Hofmann forms occurred. These organisms are clubbed, but broader and take the stain more deeply than diphtheria bacilli. The stained segments are very dark and the septa narrow and well defined, running in all cases transversely across the bacillus. They do not show any polar bodies by Neisser's method of staining, and in the few cases (5) I have examined are non-pathogenic to guinea-pigs, and do not form acid in glucose broth. Moreover in subculture they revert to the typical short form of Hofmann's bacillus with an occasional long specimen. It is only after several days' growth in subculture that many long, segmented, forms again become visible. These I term the pseudo-diphtheria form of Hofmann's bacillus (see Plate 10).

The bacilli isolated and tested by Cobbett [19] and myself may be classified as follows[1]:

I. Bacilli identical in appearance both in culture and under the microscope with diphtheria bacilli:

a. Pathogenic acid-producers, usually showing polar bodies by Neisser's method = virulent Klebs-Löffler bacilli.

b. Non-pathogenic acid-producers. Neisser's staining generally positive (?) = so-called attenuated diphtheria bacilli.

II. Bacilli somewhat resembling diphtheria bacilli but (generally shorter and) stouter:

a. Non-pathogenic, non-acid-producing segmented bacilli, showing no polar bodies by Neisser's method = pseudo-diphtheria type of Hofmann's bacillus.

b. Non-pathogenic, non-acid-producing short bacilli. No polar bodies by Neisser = typical Hofmann's bacillus.

The question whether the pseudo-diphtheria or Hofmann's bacillus is an attenuated diphtheria bacillus, capable of becoming dangerous, has been considered by various authors [19].

Roux and Yersin [85] thought they could increase the virulence of a bacillus which caused oedema but did not kill, but were unable to give virulence to a non-virulent form.

Hewlett and Knight [45] in 1897 considered that by heating they once converted a diphtheria into a pseudo-diphtheria bacillus, but were unable to repeat this, stating that "the amount of heating

[1] Founded on the classification first introduced by Park and Beebe, New York, *Med. Rec.* XLVI. 1894. Cited by Cobbett. The table is not quite the same as that given by the latter, as I have inserted (ii) A and the results of Neisser's method of staining.

Fig. 1. Hofmann's Bacillus (Pseudo-diphtheria type) 24 hour culture on serum at 37° C.

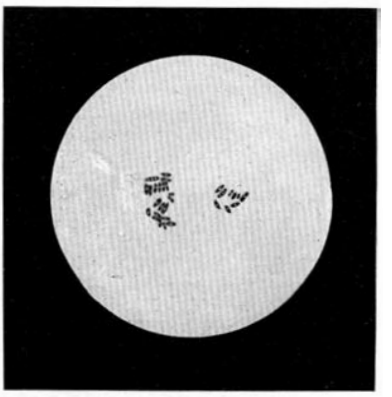

Fig. 2. The same as Fig. 1, subculture 24 hours old on serum. This represents the typical Hofmann's Bacillus. (Both figures drawn with aid of camera lucida, stained with Löffler's methylene-blue (diluted 1 : 5). Zeiss $\frac{1}{12}$ im., No. 4 oc.)

https://doi.org/10.1017/S0022172400032812 Published online by Cambridge University Press

required is a very delicate factor; too little leaves the bacilli comparatively unaltered, a little more kills them completely." They also thought that "in one or two cases" they had succeeded in transforming the pseudo into the diphtheria bacillus. They started with a typical pseudo "from the throat of a nurse who had been nursing a case of diphtheria," and cultivated it in agar for 19 generations. During this time they state it constantly altered its form, "some subcultures showing typical Klebs-Löffler forms, others typical pseudo." The 19th and 20th generations were cultivated on serum for a week, and from the latter "a broth culture was made and incubated for a week." For no better reason than this proceeding the organism suddenly became virulent. They discuss, but reject, the possibility of having started with a mixture, and on this evidence finally state: "we therefore consider that the pseudo is sometimes a modified Klebs-Löffler, though perhaps not always, as possibly more than one species having the same morphology may exist."

In 1898 Richmond and Salter[83] 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 for one case by Salter[86] in the following year. "The bacillus employed was originally obtained from a case of post-scarlatinal diphtheria." It was a typical Hofmann morphologically and was non-virulent. Its reaction in a sugar medium, one of the essential characters of the diphtheria bacillus, does not appear to have been tested. After two passages through goldfinches it was said to present a transitional appearance between a pseudo-diphtheria bacillus and a short Klebs-Löffler. After four such passages it produced oedema, but not death, in a guinea-pig, but after the fifth passage 5 c.c. killed a guinea-pig in four days with all the symptoms of experimental diphtheria. It now formed acid in neutral broth and its action was neutralized by antitoxin.

In 1902 Ohlmacher[72] published some observations on this question. He experimented with three organisms, and concluded that by a short sojourn in an immune animal a diphtheria may be converted into a pseudo-diphtheria bacillus, and that the reverse may be brought about by passing the organism through a susceptible animal. His experiments, however, only show that a long granular diphtheria bacillus after recovery from the subcutaneous tissue of a rat became short and uniformly staining, but still formed acid in glucose media. A uniformly staining, but pathogenic, bacillus after recovery from the spleen of a guinea-pig became granular, and a short uniformly staining, and slightly virulent

bacillus (killing in 7 days) after its passage through an animal became long and granular and more virulent.

Lesieur [59] could agglutinate by the serum of horses immunised by cultures of diphtheria bacilli certain varieties of this organism but not others. Hofmann's 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.

On the other hand Lubowski [63] working with non-virulent diphtheria bacilli succeeded in immunising animals and producing a serum which agglutinated not only these bacilli but also 23 different races of quite typical diphtheria bacilli. The antiserum had no action on pseudo-diphtheria bacilli.

"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 wide spread" (Cobbett [19]).

There is no evidence that bad drains and insanitary environment can ever convert non-virulent into virulent bacilli or originate diphtheria. Shattock [87] experimented on this question and found it was impossible to raise the virulence of lowly virulent diphtheria bacilli by cultivating them in a current of sewer air, even after two months.

In any consideration of this question Cobbett's observation "that in no case, as far as is known, has a virulent diphtheria bacillus been replaced by a non-virulent one before its final disappearance" from the throat, is worthy of note.

How many consecutive negative examinations are to be deemed necessary before infected persons can be released from isolation?

After deciding what types of bacilli are to be regarded as dangerous, the question as to the number of consecutive negative examinations that should be held necessary before release from isolation has to be considered.

The need for more than one negative examination has been very clearly established.

Hill [47] states that the Boston Board of Health, U.S.A., require two consecutive negative examinations of convalescents, and three for hospital patients, before they are declared free from infection.

At the South-Western's Fever Hospital, London [43], the patient is detained till the bacilli disappear as evidenced by three consecutive daily examinations.

Cobbett [18] requested the practitioners to submit swabs till three consecutive negative examinations were obtained. He found on more than one occasion that two consecutive negative examinations were followed by the discovery of the bacilli.

Out of 104 convalescent patients carefully examined at Colchester [41] on many occasions prior to discharge, one negative followed by the finding of diphtheria bacilli occurred in 11, two consecutive negatives in 10, three consecutive negatives in one, and four in one. Amongst 45 healthy infected contacts on eleven occasions diphtheria bacilli were again encountered after one negative, on three after two consecutive negatives, and once after four. In all 38 cases in which one or more negatives were followed by the finding of diphtheria bacilli. Many of these persons, both convalescents and contacts, retained their bacilli for long periods after one or more negatives had been obtained.

These misleading negatives may be due to the taking of swabs too soon after the application of some antiseptic, or to the bacilli lurking in the sinuses connected with the nasal cavities and finding their way thence into the pharynx.

Wolff [100] in 1895, examined the accessory sinuses of the nose in 22 fatal cases of diphtheria, and found diphtheria bacilli in 12, namely, once in the frontal sinus, six times in seven examinations in the sphenoidal, and twelve times out of fifteen examinations in the antrum.

Councilman, Mallory, and Pearce [26] 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 figures show that two consecutive negatives, and in some cases even three, are not a complete safeguard. In practice, however, it is occasionally difficult to enforce isolation till three consecutive negatives have been obtained, and to insist on more would be impossible. Therefore, I think, whenever practicable, a minimum of three consecutive negative examinations should be enforced before convalescent cases, or, infected contacts, are freed from isolation.

in 11 for 28 days; and in 5 for 35 days. The mean duration in these cases was therefore only about 8 days after the disappearance of the exudate.

In a later case the same author found diphtheria bacilli 49 days after the disappearance of the membrane.

Morse [68] in 25 cases found the average duration of the presence of the diphtheria bacilli after the disappearance of the membrane to be ten days.

No statement is made by the last two observers as to whether one or more negative examinations were required to prove the final disappearance of the organism.

Wesbrook [97] found the bacilli to be present in a boy for 135 days.

Woodhead [101] in his examination of the patients in the Metropolitan Asylums Board Hospitals encountered 79 cases which retained their bacilli for 100, and two for 200, days.

The varying periods during which the bacilli persist are well illustrated by the statistics on this point which have been given. The period is of course much prolonged if three consecutive, and not two, or one, negative examinations are considered necessary as proof of their disappearance. It is this uncertainty of the length of time for which bacilli may persist, and the consequent inability of the bacteriologist to state how long any patient or contact may have to be detained, which renders the enforcement of isolation difficult. The fact that the bacilli disappear, and after one or more negatives reappear, causes disappointment and irritation to the friends, and is a factor which makes the enforcement of isolation even more difficult than the actual length of time of their presence in the throat.

What class of persons should be examined and, if found to be infected, isolated?

The figures which have been already given as to the numbers of diphtheria bacilli found in "contacts" show that the percentage is highest amongst the closest contacts (*i.e.* members of an infected family), generally considerably lower amongst children in an infected school, and least in more distant contacts.

It consequently follows that in all cases the members of the family in which a clinical case has occurred ought to be examined, and, if the patient is a child attending school, at least the other members of his or her class. It also is most certainly advisable, whenever the circumstances render it at all possible, to follow up and examine other

children who may have played with the patient or otherwise have come in contact with him.

In epidemic times all suspicious, and if possible all, cases of sore throat should be subjected to examination, especially if amongst scholars of infected schools.

Persons who were supposed to be suffering from tonsilitis have on several occasions been found to be infected with diphtheria. These are not only a danger to the community but are liable to suffer from the effects of toxaemia, owing to the omission of antitoxic treatment. Cases are cited later in which such persons have been the means of spreading the disease unknowingly.

Patients suffering from affections of the nose, such as membranous rhinitis[1], have frequently been demonstrated to be the carriers of infection.

Faucial diphtheria has only seldom, however, been observed to have been contracted from cases of membranous rhinitis, though instances are recorded by Cobbett (18), Dowson (30), Park (75), and Ravenel (82). On the other hand a case of membranous rhinitis has not unfrequently been observed to give rise to another of the same kind. Examples have been cited by Abbott (1), Concetti (25), Dowson (30), and Ravenel (82); and Lieven (60) "reported one case from which he obtained an organism that when introduced into the noses of other children by means of tampons caused a similar disease in them."

Ravenel (82) collected 41 cases of membranous rhinitis in which there was a record of bacteriological examination. In 33 of these diphtheria bacilli were found. In about 20 % of cultures from cases of this disease tested for their pathogenic action on guinea-pigs, the virulence has been proved to be low, and moreover it has frequently been observed that the cultures rapidly die. The result of the examination of 52 cultures for virulence is given below.

	Virulent	Attenuated
Abbott (1)	2	1
Baginsky (5)	1	0
Cobbett (18)	1	0
Concetti (25)	2	0
Dowson (30)	2	0
Lack (57)	23	0
Park (75)	0	5
Ravenel (82)	5	3
Stamm (89)	3	0
Townsend (91)	4	0
	43	9

[1] See note on membranous rhinitis. Cobbett, *Journ. of Hygiene*, Vol. I. No. 2, p. 232. Also Hunt (50).

Symes[90A] has recently observed diphtheria bacilli in 20 (87 %) out of 23 cases of atrophic rhinitis; morphology in culture was principally relied on. 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 examined by him of cases of ozoena, 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 sharply distinguish between the diphtheria and Hofmann's bacillus, saying that among the normal noses in 58 % " a short diphtheria-like or pseudodiphtheria type of bacillus was present in the nose."

In a few instances diphtheria bacilli have been discovered in conjunctivitis (Jessop[51], Stephenson[90], Eyre[33] and others), and in lesions of the skin (Gordon Sharp[40], Park[76], Townsend[91], Wright[102], and Müller[69]): but in both these situations diphtheroid bacilli have frequently been encountered. In the conjunctiva the Xerosis bacillus, whose relationship with the diphtheria bacillus I do not propose to discuss, has been investigated by many observers (Berger[7], Eyre[33], Lawson[58]), and organisms morphologically resembling diphtheria bacilli have been isolated also from various lesions of the mucous membranes, *e.g.* catarrh of the cervix uteri, cancrum oris, urethritis, and pyorrhoea of the gums (paper by Fullerton and Bonney[35] and discussion on it). In consequence of these facts all organisms resembling diphtheria bacilli found in lesions of the skin and mucous membranes must be thoroughly investigated before their identity with the diphtheria bacillus can be established.

Also in times of epidemics it would seem most desirable to follow up any group of cases in which the evidence points to a common source of infection, as by the purchase of articles of food at certain shops, and the persons engaged in supplying or making these articles and their families should be examined.

In regard to the selection of infected persons for isolation some of the recommendations of the Massachusetts Committee[65] might be followed with advantage; these have already been cited. Briefly, they advise the isolation of infected children, and persons dealing with children; the exclusion from work of infected persons trading in articles of food; but not the isolation of bread-winners. They suggest

that these latter should be warned of the danger they are to the public and instructed in the use of antiseptic mouth-washes.

The question of notification of infected contacts is discussed and answered in the negative by Cobbett [20].

How are the diphtheria bacilli communicated by infected persons to others?

The majority of the authors who have been already quoted strongly emphasize the danger to the health of the community by healthy infected persons, and occasionally cite examples.

In epidemic times, though of necessity many instances must have come to their knowledge in which the probable channel of infection could be traced, considerable difficulty is often met with in confirming these cases and excluding all other possible sources of infection.

I have therefore thought that some of the instances detailed in recent literature might with advantage be quoted since one authentic example carries more weight than the mere quotation of abstract opinion.

Bisset [10] 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 states that many such fully authenticated examples have occurred in the city of Buffalo.

Burnett [14] mentions an interesting case. In a school a boy was supposed to be suffering from a severe cold. There was a nasal discharge, which had been observed to be staining the pillow since the first day of term. On bacteriological examination diphtheria bacilli were found. No other case of catarrh in the school had these organisms. After the discovery every boy and master (with one exception) was given a prophylactic dose of antitoxin. The master who had not been given the dose contracted the disease, but no one else.

The bacilli lingered in this boy's throat for three months in spite of treatment. Finally after three consecutive negative examinations he was allowed to return to school when the term commenced; but the master was kept away, as after one negative the bacilli were again found. Soon after the beginning of term a fatal case of diphtheria occurred, when the boy's throat was again tested and diphtheria bacilli were found in it, and in the throats of three others.

Auden [4] attended a child suffering from diphtheria eight days after birth. Diphtheria bacilli were isolated. The mother said she had had a very severe sore throat one month before the child was born which had continued to cause difficulty in swallowing until one week before confinement. No external source of infection could be discovered.

Peck [80] gives a good instance of the transmission of the disease.

A boarding-school had 50 boarders and 50 day scholars. On October the 5th two of the boarders had sore throats. Swabs were taken and diphtheria bacilli discovered. The day school was 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 the 8th. All persons in the house were then examined with negative results except two. The three cases and two contacts were then isolated.

On Nov. 5th all the scholars reassembled and all were bacteriologically free. A. B. returned on Nov. 12th, but went home on Nov. 16th, and on Nov. 19th developed diphtheria. It was then ascertained that she had not been examined, but had been using an antiseptic spray. On Nov. 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.

The incubation period in A. B.'s case was 5 weeks.

White [99] states that a child in a tenement house suffered from diphtheria, and cultures revealed virulent diphtheria bacilli for three months. After one negative the child was released from isolation, but two days later cultures from the throat of this child and two others, who had been in contact with him, showed diphtheria bacilli, although the latter were never ill. Two other children coming to the house were exposed to the latter for two days and then returned home. In five days one of these developed diphtheria. Other sources of infection were excluded.

Cobbett [18] gives an example in which the distribution of infection was traced to a boy suffering from chronic membranous rhinitis, in the discharge from which were many virulent diphtheria bacilli. All the members of the family also had diphtheria bacilli. The boy had been attending school during three weeks in this condition. Six out of eight boys in his class suffered subsequently from the disease.

I [41] have given at length an account of a small outbreak in a school probably introduced by a child suffering from what was regarded as only a mild sore throat.

Park [76] traced a group of cases of diphtheria to a candy-store kept

by a family in which a case of diphtheria had occurred. Children who bought candy at the shop acquired diphtheria, and other children, who came in contact with the healthy children of this family at school, also developed diphtheria.

Two children in a milkman's family were found to be suffering from diphtheria; the other members of the family were examined with negative results. Three weeks later diphtheria began to make its appearance amongst the milkman's customers. The two men employed in milking the cows were finally examined and found to be harbouring virulent bacilli. (Denny [29].)

The dangers to public health attendant on the free communication of infected contacts with normal persons are well brought out by these instances.

The ways in which infected persons may communicate their bacilli to other persons are very numerous.

The kissing of babies and children as a means of spreading the disease has been insisted on by Hewlett and Murray [46].

In schools, however, probably sweets, pencils, pens, slates, &c. which pass from one child to another, and especially the habit children have of placing their fingers, and such articles as pencils, in their mouths may explain the rapid spread of infection in such institutions; and the absence of such habits in adults may to some extent account for their relative immunity from the disease.

Cobbett [18] traced the spread of the disease amongst certain children in a class to the hours during which slates were used.

In this connection Bond [11] remarks that he has ascertained that in certain schools each child does not have its own slate, and that as a method of cleaning their slates licking is common.

Cobbett also gives an excellent illustration of the dissemination of the disease by means of pencils. A boy, the day before he was taken ill of diphtheria, spent the evening with some neighbours. Four of the latter were examined, and diphtheria bacilli found in two boys, but not in a baby and a girl. On inquiry it was discovered that the original boy and the two others had played at parlour cricket, and each had taken it in turn to score with the same pencil which often, doubtless, found its way into their mouths.

The results of such contact in schools can only be prevented by the isolation of the infected, the systematic disinfection of the various articles in general use, the limitation of the use of certain articles to each child, and by bringing to the knowledge of the teachers the

possible ways in which the disease may be spread, as well as the importance of supervision and cleanliness. In some countries the use of slates in schools has been prohibited on hygienic grounds.

Can Infection be carried otherwise than by Infected Persons?

The answer to this is undoubtedly in the positive; but as compared to the method of dissemination by personal contact the means is probably rare.

Instances of contagion caused by *milk* are not uncommon—one has already been cited.

Bowhill [12] in connection with an outbreak of diphtheria at Cardiff attributed to infected milk, isolated from the suspected milk a diphtheria bacillus, whose virulence for guinea-pigs was proved by Nuttall.

Klein [55] found a typical, and pathogenic, diphtheria bacillus in one sample of milk out of 100 examined. Careful inquiries failed to show its origin, or that any persons had acquired the disease by drinking the milk. He [53] has also made the important observation that these organisms multiply rapidly in stored milk.

Eyre [31, 32] also isolated the bacillus from a sample of milk, and subsequently made observations on 5 organisms derived from milk resembling the diphtheria bacillus in appearance, but not in pathogenicity.

Dean and Todd [28] found virulent diphtheria bacilli in certain ulcers on the teats and udders of cows and in their milk. One child which drank this milk developed diphtheria, and some other persons had sore throats, probably diphtherial. They proved by experiment, however, that the lesions on the teats were probably due to a separate infection on which the diphtheria bacilli were superadded.

Littlejohn [62] in his report on the health of the city of Edinburgh stated that it was free from diphtheria at the end of May 1900. On May 29th there was 1 case, during the next week 30, and the week following 40. The milk supply in this district was mainly from one dairy. The dairy-keeper and his family had sore throats in which diphtheria bacilli were found. The milk supply was stopped, and the epidemic ceased.

Howard [48] quotes several milk epidemics, and investigated one at Ashtabula, Ohio. He failed to find the bacilli in the milk or mouths of the dairymen, but ascertained that the son of one of the latter had lately been suffering from a very severe sore throat.

Klein [53] in 1889 inoculated cows subcutaneously with diphtheria cultures and found that they suffered amongst other lesions from eruptions on the udders and teats. From the latter lesions and the milk (drawn off) with precautions against infection from them, and from the seat of inoculation he was able to recover the bacilli. Subsequently he carried out other experiments of a similar nature.

Abbott [2] repeated these experiments, but was unable to confirm Klein's observations either as to the eruptions on the udder, or as to the appearance of the bacilli in the milk. Ritter [84] similarly failed to show that they passed into the milk.

Klein [54] replied to Abbott's criticisms of his experiments, and pointed out the causes for his failure.

Carstairs [16] recounts a case of a father and son who were cornet-players being attacked by diphtheria. The instrument was put away. Four years later a younger member of the family having found the cornet played it and developed diphtheria in a week. There had been no other case in the district for eight months previously.

Trevelyan [92] gives an instance in which diphtheria bacilli were cultivated from a handkerchief, eleven weeks after it had been used by a child suffering from diphtheria.

Vincenzi [93] examined the holy water in churches during an epidemic, and found, amongst other bacteria, diphtheria bacilli. He demonstrated their presence by culture and by virulence tests.

At Colchester, as a precautionary measure, the cups were removed from the public drinking-fountains, and a little later the water was cut off to prevent the children drinking from the spouts.

Weichardt [95], on the other hand, examined various objects, such as walls, linen, &c. about diphtheria cases by means of damp swabs. 300 samples were collected from 50 parts of the sick room, and 250 from other parts of the house (22 rooms in all). Diphtheria bacilli were found only three times, and in each case from objects which had been in direct contact with the child's mouth.

Welch [96] showed that in many examinations of hospitals diphtheria bacilli were not discovered except in situations which had been infected by direct contact with the patient, or his discharges, and that the bacilli were not present in the air.

The diphtheria bacillus has also been discovered by Cobbett [22] in the *horse*. Its cultural peculiarities and virulence were fully tested.

The daughter of the owner was suffering from diphtheria and the condition was only brought to notice on this account.

Bacilli morphologically identical with the diphtheria bacillus have been described in chickens with a contagious disease called "roup" by Gordon Sharp[39] and also by Gallez[36]. The former points out some connections between the epidemic in chickens and the disease in man, but he thinks that the bacilli in fowls are less virulent than in man.

It should be pointed out however that diphtheria-like organisms have also been discovered in pigeons, both in normal individuals and those suffering from "pigeon canker." They produce acid in glucose broth and stain by Neisser's method, but are non-pathogenic for guinea-pigs (Macfadyen and Hewlett[64]). The disease known as avian diphtheria in France, which appears in very fatal epidemics in fowls, is however due to a completely different organism (Guérin[42]).

Klein[53] experimented on *cats*, and found that they succumbed to inoculations of diphtheria bacilli. Some of these animals suffered from respiratory troubles, others from paralysis and weakness, but all, at autopsy, showed marked changes in the kidneys, which were enlarged and had extensive areas of fatty degeneration in the cortex. He also found that certain cats accidentally fed with milk from the inoculated cows acquired the disease and transmitted it to others. He further fed cats experimentally on milk containing diphtheria bacilli, and found that they died of a similar disease, but in none of these cases did he apparently cultivate the organism from those animals. Abbott and Welch[3] by tracheal inoculations showed that kittens developed a pseudo-membrane in the larynx, and died suffering from respiratory trouble and great weakness.

Klein[53] cites an interesting case, reported by Dr Bruce Low, of the spread of diphtheria by a cat. "A little boy had a fatal attack of diphtheria. On the first day he vomited, and the cat licked up the vomit on the floor. In a few days (and after the death of the boy) the cat was noticed to be ill, and her sufferings became so severe and similar to those of the dead boy that her owner destroyed her. During the early part of its illness this cat was let out into the back yard; a few days later the cat of a neighbour who lived a few doors off was noticed to be ill. This cat had also been in the back yard at night. This second cat recovered, being carefully nursed by four little girls, all of whom developed diphtheria. There was no other known source of infection to which these girls had been exposed except the cat." He also enumerates several other cases of a similar description.

Diphtheria-like bacilli have also been isolated by Dowson [30] from cats suffering from illness during an epidemic of diphtheria at Bristol. These showed the kidney lesions described by Klein.

Although in the majority of instances the disease would not be likely to spread by infected animals yet the possibility of their conveying the bacilli, and of causing isolated cases, must be borne in mind.

The question as to how certain persons can harbour virulent diphtheria bacilli without being ill has been answered by the researches of Wassermann [94], Orlowski [73], and others. The former found considerable quantities of antitoxin (enough in 1 c.c. of serum to protect guinea-pigs completely against 10 fatal doses) in the blood of 50 % of children and 83 % of adults in 17 and 28 examinations respectively; and the latter found that 5 out of 10 children in a hospital had antitoxin in their blood. None of these persons as far as could be ascertained had suffered from diphtheria.

Fischl and Wunscheim [34] also found more or less antitoxic substance in the placental blood of 68 out of 82 infants (83 %); and Cobbett [23] found this substance in the blood of 8 out of 11 horses.

It is probably such immune persons who carry the bacilli and give rise under favourable conditions to epidemics.

The enumeration of the foregoing facts and the deductions based on them render it most desirable that further studies should be made into the ways in which the disease spreads, and the occurrence of diphtheria bacilli in healthy persons who have not been exposed to infection. It is for this reason that I have ventured to give the results of certain investigations on the occurrence of Hofmann's bacillus in the healthy throats of contacts and others, and the bacteriology of the mouths of normal persons (non-contacts).

Methods.

Swabs were prepared, constructed of cotton-wool and wrapped round a stout wire. These were placed inside stout glass test-tubes and sterilized.

In obtaining a culture for examination the throat, or nose, of the person was wiped with the swab, which was then returned to its case. As soon as possible the infected swab was rubbed over the surface of a serum tube[1]. The culture so obtained was grown at 37 % for 24 hours or less.

[1] See footnote on p. 229.

At the time of examination samples from dissimilar colonies were streaked on coverslips by means of a sterilized platinum needle, stained by Löffler's methylene-blue (diluted 1 : 5), mounted in the stain and examined under a $\frac{1}{12}$ oil immersion lens (see Cobbett and Phillips [24]). Unless the growth was very scanty, or the culture was very thickly studded with colonies, organisms from more than one colony were never placed on the same portion of the coverslip. By this means the appearance of the various colonies and the morphology of the bacilli derived from them could be studied, and the difficulties in distinguishing the organisms in a general smear avoided.

In all cases in which suspicious bacilli occurred ·5 % acetic acid was run under the coverslip in the way suggested by Cobbett [21], or a separate preparation from a similar colony was stained by Neisser's method. In many cases the two methods were compared.

By these methods of staining it was found that in almost every case diphtheria bacilli were distinguishable by the presence of darkly stained polar bodies; whereas Hofmann's bacilli had none. It is true that certain examples of the diphtheria bacillus showed no polar bodies[1] by the acetic acid or Neisser methods, and that inconspicuous and scarce polar bodies were sometimes seen in Hofmann's bacillus. Also certain bacilli[2] and cocci were encountered which showed polar bodies by both these methods. These facts do not, however, materially detract from the value of the method, which is a considerable aid to diagnosis, so long as it is not trusted to alone.

Cobbett [41] had previously come to the same conclusion. Beaton, Caiger, and Pakes [6] have experimented with Neisser's stain and are convinced of the utility of the method, and Cammidge [15] agrees with them.

Hewlett [44] considers that "with precaution to exclude fallacies, and using fresh membrane, Neisser's method will often afford a means of rapid diagnosis."

Besides the tests which have been already given, a large number of pure cultures of both diphtheria and Hofmann's bacilli were grown in glucose broth. The former without exception formed acid, the latter did not.

[1] The absence of polar bodies is not an indication of want of virulence—one example of the diphtheria bacillus which neither in the original, nor subsequent, cultures showed them was virulent.

[2] I hope to call attention later to the occurrence of polar bodies in certain other organisms resembling diphtheria bacilli when treated by these methods.

A few examples of each were tested for virulence. The results were exactly in accord with those of Cobbett, namely, a small portion of diphtheria bacilli were found to be non-pathogenic, but the majority were pathogenic in doses of ·1 c.c. Hofmann's bacillus was not found to have any pathogenic properties, whether the typical, or pseudo-diphtheria, forms were tested.

The pseudo-diphtheria form of Hofmann's bacillus is one of the few organisms met with in Cobbett's experience, or my own, which is often likely to be mistaken for the diphtheria bacillus. Even when these are present I completely agree with Cobbett when he says, "it is possible to train the eye to distinguish with a sufficient degree of precision the diphtheria bacillus from the bacillus of Hofmann" (see p. 229).

The want of accord in the positive and negative diagnoses of cultures given by the various bacteriologists who made observations for the Massachusetts Committee[65] as compared with the Committee's standard seems to mostly depend on whether, or not, they considered Hofmann's bacillus in its various forms to be a causal agent in diphtheria.

Most of the other observers who have been quoted give statistics detailing the number of times they have found this organism and appear to attach some importance to its presence. Pakes[74], for example, thinks it capable of giving rise to a specific sore throat.

Cobbett on the other hand, as has already been seen, comes to the conclusion that it is not in any way connected with disease in man. He says[20] "my experience of the outbreak of diphtheria at Cambridge (1900) gave no reason for thinking that the pseudo-diphtheria bacillus (Hofmann) is other than perfectly innocuous to man." And his observations in the Spring epidemic of 1902 confirmed these views. I have found this bacillus in a large number of cases, and agree with him both in the above opinion and in his statement that it is a common inhabitant of the mouths of the poorer classes.

Its non-occurrence to a great extent in the mouths of patients suffering from diphtheria is, I believe, to be explained by the fact that during epidemics, once the diphtheria bacillus has been found, no further search is made, but when the latter organism is disappearing a prolonged search is often necessary, and in the course of it the bacillus of Hofmann is encountered and recorded. Hence the view is likely to be entertained that it is an attenuated form of the diphtheria bacillus. Cultures of this organism from persons in every stage of convalescence

from diphtheria have been isolated by Cobbett[19] and all were found to be equally harmless.

Regarding the bacillus of Hofmann as a common innocuous inhabitant of the mouth I think its prevalence can be made to illustrate the manner in which diphtheria bacilli are carried from mouth to mouth.

During the outbreak at Colchester I examined 576 cultures from non-infected contacts of all classes and found the bacillus of Hofmann on 316 occasions (54·8 %). The great majority of the persons examined belonged to the poorer classes, and the bacillus was of very frequent occurrence in their throats, whereas in the more well-to-do persons the percentage of cases in which it occurred was much lower.

In schools attended by the children of the poor where many articles are shared in common, and want of strict attention to cleanliness is frequently observed, 64·5 % harboured this bacillus (411 examinations).

The following table gives the percentage occurrence of various common organisms in the mouths of non-infected contacts amongst school-children and others.

Class of persons examined		No. of cultures	Percentage of Hofmann	Staphylococci	Streptococci	Oval bacilli	No. of healthy infected persons not included in Nos. given	
Scholars: School	I	6	**66·6**	16	0	0	0	These schools were attended by the children of the poor. 411 cultures examined. Hofmann's bacillus found in 64·5 % of examinations
,,	II	50	**64**	56	2	2	8	
,,	III	49	**63·3**	49	0	2	8	
,,	IV	149	**63**	67	2	30	6	
,,	V	59	**62·7**	49	4	2	5	
,,	VI	16	**62·5**	44	6	13	1	
,,	VII	15	**60**	60	0	7	5	
,,	VIII	37	**57**	66	0	9	5	
,,	IX	30	**56·6**	53	0	0	0	
,,	X	9	**33·3**	67	0	11	1	Better class schools. 19 examinations. Hofmann bacillus found in 31·5 %
,,	XI	10	**30·0**	90	0	0	0	
Persons above school age		40	**50**	43	8	5	3	
Persons above 20 years of age		13	**30·7**	61	8	8	2	Included in above list. Mostly parents of scholars in schools I—IX
Below school age		14	**42·8**	79	0	0	0	
Well-to-do persons		79	**22·7**	68	8	12	23	

The above table shows how very commonly the bacillus of Hofmann is found in the throats of non-infected contacts. Among the scholars in

the poorer schools its range is from 66·6 to 56·6 %. Here the opportunities for spreading are very great by the various methods that have already been described. Amongst older and younger persons of the same class who are liable to acquire the organisms in similar ways, but not to the same extent, its range is from 50—42·8 %. Thirteen persons, the parents of these children, harboured it only in 30 % of cases.

In the rather better class schools it is present in much smaller numbers, namely, in 33 % and 30 % of individuals (only 19 persons however were examined), and amongst the well-to-do class only 22·7 % were found to harbour this organism in their mouths.

The latter, moreover, were in nearly all cases closely related to diphtheria patients, and consequently a large number of infected contacts were found amongst them.

In order to ascertain to what extent Hofmann's bacillus was present amongst the members of poorer families, where the children often attend different schools, the following table was constructed.

Families not infected with Hofmann's bacillus		Families in which Hofmann's bacillus was found in at least one member		Families with every member infected with Hofmann's bacillus		
No. of persons per family	No. harbouring Hofmann	No. of persons per family	No. harbouring Hofmann	No. of persons per family	No. harbouring Hofmann	
6	0	5	4	8	8	
5	0	5	1	4	4	
4	0	4	3	4	4	
3	0	4	3	3	3	
3	0	4	3	3	3	
3	0	4	2	3	3	
3	0	4	2	3	3	
3	0	4	1	3	3	
2	0	3	2	3	3	
2	0	3	2	2	2	Total percentage of persons harbouring Hofmann's bacillus in families infected with this organism 73·6 %. 66 families examined.
2	0	3	2	2	2	
2	0	3	1	2	2	
2	0	3	1	2	2	
2	0	3	1	2	2	
2	0	2	1	2	2	
2	0	2	1	2	2	
2	0	2	1	2	2	
2	0	2	1	2	2	
2	0	2	1	2	2	
2	0	2	1	2	2	
2	0	2	1			
2	0	2	1			
		2	1			
		2	1			
58	0 (0 %)	72	38 (52·8 %)	56	56 (100 %)	

https://doi.org/10.1017/S0022172400032812 Published online by Cambridge University Press

The results are somewhat remarkable, and show that when the organism has once been introduced into the family many of the younger members acquire it. Dr Cobbett very kindly placed at my disposal his records of the results of his examinations. I find that he has noted the organisms discovered in 1495 examinations in which diphtheria bacilli were not found. He recorded the presence of Hofmann's bacillus in 35·9 % of these observations.

His records[1] resemble mine to a striking degree as far as the distribution of this bacillus in school-children and well-to-do persons is concerned. Its range in three poor schools was from 78·9—63·3 %, whereas amongst the undergraduates of Sidney Sussex College, Cambridge, it only occurred in 21·9 % of individuals.

To the following table giving Cobbett's results I have added those of the recent examinations of two schools by myself.

Class of persons examined	No. of cultures	Percentage				
		Hofmann	Staphylo-cocci	Strepto-cocci	Oval	Healthy contacts
Non-infected contacts	1495	**35·9**	65	24	5	?
Scholars:						
Poorer school I	19	**78·9**	84	21	15	1
,, ,, II	49	**75·5**	38	2	0	0
,, ,, III	120	**63·3**	62	13	2	8
*Better class school IV†	29	**0**	66	10	17	0
* ,, ,, V	49	**12·2**	71	25	39	1
Undergraduates of Sidney Sussex College	41	**21·9**	78	20	10	3

* Examined by me, 1902. † School situated in the country.

In a total of 2198 examinations of non-infected contacts by Cobbett and myself the bacillus of Hofmann was found to be present on at least 870 occasions (nearly 40 %).

It was found comparatively infrequently in the mouths of well-to-do persons (13·3 % of 217 examinations), and very commonly amongst the poorer children (65·7 % of 599 examinations).

The vast majority of these persons were in perfect health, but a few had sore throats at the time of examination. So far as I can ascertain none suffered from diphtheria within several months.

[1] At the time of the various examinations made by Dr Cobbett and myself the organisms observed were recorded, but no idea of classifying them was at the time entertained. Except in the case of Hofmann's bacillus the percentages given are probably too low, since every class of organism present was not always recorded.

I think the following deductions may be drawn from these statistics.

(1) That the bacillus of Hofmann (as previously defined) is perfectly innocuous to man.

(2) That it is a common inhabitant of the mouths of the poorer classes, especially children.

(3) That it is relatively uncommon amongst well-to-do persons, and even amongst the children of this class.

(4) That it probably spreads from one child to another by the means, that have been indicated as the probable ones, by which diphtheria bacilli are transferred from one individual to another.

(5) That in the absence of diphtheria bacilli morphologically resembling Hofmann's bacillus, described by Wesbrook in an outbreak at Owatonna, but which have never been met with by Cobbett or myself, no importance whatever should be attached to the presence of Hofmann's bacillus.

Organisms found in the mouths of healthy persons who have not been exposed to the disease.

In view of the satisfactory results which have followed in those outbreaks in which strict isolation of convalescent patients and healthy infected contacts has been enforced, and the theoretical and practical importance of ascertaining whether virulent diphtheria bacilli are to be found in the mouths and noses of healthy persons, who have had no opportunity of acquiring these bacilli by contact with the disease, I have tabulated the results of examination of some of these persons.

It has been pointed out earlier that when investigations of this kind have been carefully conducted, and measures taken to test the virulence of organisms, apparently identical with the diphtheria bacillus, which have been discovered, and also to inquire into the history of persons from whom virulent diphtheria bacilli have been isolated, the proportion of cases in which inquiry did not lead to the discovery of recent contact was very small, namely, 3 (·26 %) in 1149 persons examined.

Remembering the great difficulty often met with in prosecuting inquiry amongst 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 seldom, if ever, exist in the mouths of the normal population.

In the following tables are given the results of the examination of 362 persons, several of whom were examined more than once. Some were workers in the pathological laboratory; some undergraduates in the University; some patients in Addenbrooke's Hospital; but the larger number were persons of the poorer classes, suffering from sore throats, and their friends.

Swabs were sent up for examination from a few of the latter persons by medical practitioners in attendance, but in the majority of cases an examination was only made because I desired to ascertain what organisms were present. The patient was usually able to procure swabs from friends also. In every instance here given inquiries were made as to any possibility of contact with recent diphtheria cases, or healthy infected contacts, and only cases, in which no probability of such an occurrence could be ascertained, are inserted here. In the few cases in which the history pointed to a possibility of contact the results have not been recorded, though all were negative.

All these persons may then be regarded from our point of view as normal individuals.

In this series a considerable proportion of the persons examined were above the age at which diphtheria is most common. This is to be regretted; but I hope at a later date to be able to record the results of examinations of healthy children.

In no single instance amongst these 362 persons was a virulent diphtheria bacillus met with, nor any organism that could not be distinguished morphologically and by staining methods, with the exception of one non-virulent diphtheria bacillus.

The few organisms which slightly resembled diphtheria bacilli were isolated in subculture, and tested for their power of producing acid in glucose broth, and for virulence.

In view of certain observations which have been quoted it is most desirable that as far as possible in all investigations on healthy throats the virulence of all organisms morphologically and culturally resembling diphtheria bacilli should be tested; and that when virulent bacilli are found, the effects of simultaneous injection of antitoxin should be ascertained[1].

[1] I have lately found an organism resembling the diphtheria bacillus in its morphology. It forms acid in glucose broth and gives a positive Neisser reaction, and kills guinea-pigs in 24 hours. On post-mortem examination however the organisms were cultivated from

Since the diphtheria bacillus acts both on man and guinea-pigs by means of its toxine, and non-virulent bacilli produce no toxine in culture, without definite proof to the contrary it may be inferred that the non-virulent diphtheria bacillus is harmless to man. At present this appears to be the opinion of most authorities.

Table showing the organisms found in the mouths of healthy non-contacts.

	Persons examined	Hofmann's bacillus	Staphylococci	Streptococci	Oval bacilli	Diphtheria bacilli
Members of the University	48	**4·1 %**	77 %	16·6 %	27·0 %	0
Patients in Addenbrooke's Hospital	98	**12·2**	63·3	8·1	30·5	1
Workers in the Pathological Laboratory	18	**22·2**	44·4	55·5	66·6	0
Other persons	198	**24·7**	77·7	13·1	22·2	0
Total	362	**18·5**	72·9	14·8	27·3	·27

As has already been stated most of these persons were adults, who are apparently not so liable as children to acquire Hofmann's bacillus, since daily congregations of individuals as in schools do not occur, and the habit of placing various articles in their mouths is not so common as amongst children. The table, however, shows that nearly a quarter of persons of the poorer class, placed under the last heading, harboured these organisms in their mouths, while but 4 % of the members of the University did so. It is also seen that the hospital patients, who belonged to the same class as the former, harbour them only to the extent of 12 %. This seems to be due to the fact that the majority of the latter were country people, and consequently less likely than persons of their own class in towns to acquire bacilli by contact.

The one example of the diphtheria bacillus, and that a non-virulent one, was met with amongst the hospital patients. No history of contact could be obtained.

On combining these observations with those of the other workers mentioned (page 219) who have made inquiries as to the possibility of recent contact and worked out the virulence of the organisms they observed, it is found that amongst 1511 persons virulent diphtheria bacilli were only isolated on two occasions, and non-virulent on thirty.

the heart's blood and organs, and numerous microscopic haemorrhages crowded with bacilli were found on section. In this respect, and in the appearance of its colonies, it differs from the diphtheria bacillus. This is apparently the organism described by Davis[27] in 1898.

I have also found in the mouth diphtheroid organisms resembling those described by Foullerton and Bonney[35].

Also in one case bacilli morphologically identical with diphtheria bacilli, but too few to be subcultured, were seen. Including the doubtful one the percentage of virulent diphtheria bacilli met with amongst these persons is ·19, and of the non-virulent 1·98.

SUMMARY.

1. Diphtheria bacilli have been found in a considerable proportion of persons who have come into contact with cases of diphtheria or with other infected persons.

2. Such persons have been shown to be a grave danger to public health, especially when frequenting schools or institutions, and to constitute the usual channel by which the disease is spread.

3. Very satisfactory results have followed on the isolation of convalescents from the disease and of infected "contacts," where two or more consecutive negative examinations have been required before release.

4. Carefully conducted investigations amongst healthy persons, who have not at a recent date been in contact with diphtheria cases or infected "contacts," have shown that virulent diphtheria bacilli are very seldom (3 examples amongst 1511 persons) present in the mouths of the normal population. This fact renders the discovery and isolation of infected persons a practicable possibility and offers a fair prospect of discovering and isolating the majority of them in any outbreak.

5. Diphtheria bacilli are usually distinguishable on morphological and cultural grounds, but whenever possible it is desirable that their virulence should be tested.

6. The bacillus of Hofmann is innocuous to man, and is a very common organism in the mouths of the poorer classes. The distribution of this bacillus points to the conclusion that it is carried from mouth to mouth in the same way as the diphtheria bacillus, and therefore its widespread prevalence in schools attended by poorer children is significant, as showing how widely spread and uncontrollable an outbreak of diphtheria may become unless measures are early taken to deal with infected contacts.

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