A STUDY OF THE VIRULENCE OF THE DIPHTHERIA BACILLI ISOLATED FROM 113 PERSONS, AND OF 11 SPECIES OF DIPHTHERIA-LIKE ORGANISMS, TOGETHER WITH THE MEASURES TAKEN TO CHECK AN OUTBREAK OF DIPHTHERIA AT CAMBRIDGE, 1903.

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DURING the spring of 1903 an outbreak of diphtheria occurred amongst the children attending certain schools in Cambridge. The measures taken to check this outbreak included the bacteriological examination of all notified persons and contacts, and the isolation of such as were found to be harbouring diphtheria bacilli in their throats. These measures depend on the observation that a certain number of persons, who have come into contact with cases of diphtheria, become infected with the specific organism, and, while remaining healthy, are liable to give the disease to others. The bacteriological efforts to check the disease are also based on the assumption that *virulent* diphtheria bacilli do not occur in the mouths and noses of healthy people, 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 some 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 infected individuals is not only likely to prove unsuccessful, but entails unnecessary hardship on the isolated persons.

Before proceeding to the results of measures adopted in this instance, a short summary of some of the investigations, on which the principles depend, is given.

The occurrence of diphtheria bacilli in notified persons[1].

The results of the bacteriological examination of nearly 27,000 persons certified on clinical grounds to be suffering from diphtheria by English, Continental, and American investigators show that in 72 % organisms morphologically resembling diphtheria bacilli are present. Virulence and cultural tests of the bacilli found have only been made in a very small proportion of cases.

[1] For a more detailed account of the investigations summarized in these paragraphs see Graham-Smith (1903, pp. 217—219), and the original papers there referred to.

The occurrence of diphtheria bacilli in persons who have come in contact with cases of the disease, or with others who have acquired the bacillus in this way[1].

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

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.

Table I gives the results of several investigations on this subject.

TABLE I.

Name of observer	No. of persons examined	No. of infected persons found	Percentage of infected persons	
Infected Families.				
Cobbett (IV. 1901, p. 231)	9?	9?	100	
Spirig (1899)	9	6	66·6	59
Park and Beebe (1894)	48	24	50	
Hospital Wards.				
Lister (1898)	125	61	48·8	
Müller	100	14	14	23·4
Park and Beebe (1894)	55	6	10·9	
Chatin and Lesieur (1900)	75	2	2·6	
Schools.				
Goadby (1900)	600	190	34·1	
Peck (1901)	100	31	31	
Berry and Washbourn (1900)	142	17	11·9	20·1
Denny (1900)	200	22	11	
Graham-Smith (1902)	519	54	10·4	
General Contacts.				
Meade Bolton (1896)	214	95	45·5	
Kober (1899)	128	15	11·7	13
Cobbett (IV. 1901, p. 242)	650	19	2·9	

The figures which have just been quoted emphasise the fact that a very considerable proportion of the persons exposed to the disease

[1] See note p. 259.

acquire diphtheria bacilli without signs of illness. The proportion is highest amongst the closely related persons in the infected family, and gradually diminishes as the opportunities of contact become less frequent.

The occurrence of virulent diphtheria bacilli in persons who have not recently been in contact with the disease[1].

Reliable observations on this point are very few, since most of the investigators have not isolated and tested the organisms they have found, but have depended on their morphology in culture alone, nor have they usually made any special inquiries into the possibility of recent contact.

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

The results of observations, in which morphology in culture alone was in most cases relied on, are given in the following table.

TABLE II.

Observer	No. of persons examined	No. of persons in whom organisms morphologically resembling diphtheria bacilli were found
Herman Biggs	330	32
Garratt and Washbourn (1899)	666	8
Hewlett and Murray (1901)	385	58
*Massachusetts Committee (1902)	1993	10
	3374	108 (3·2 %)

* In their table the authors of this report include persons examined at Boston, New York and Brooklyn with the places from which came the persons here recorded. Owing to the statement that diphtheria was prevalent in these cities I have excluded them.

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

[1] See note p. 259.

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, and all suspicious organisms isolated and tested for virulence.

TABLE III.*

Observer	No. of persons examined	No. of persons harbouring non-virulent diphtheria bacilli	No. of persons harbouring virulent diphtheria bacilli
Park and Beebe (1)	324	24	2
Kober (1899)	590	5	0
Denny (1900)	235	1?	0
Graham-Smith (1903, p. 252)	362	1	0
	1511	31 (2·05 %)	2 (·13 %)

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

The investigations of these observers have shown that in a large proportion of their cases, in which bacilli morphologically and culturally identical with diphtheria bacilli have been found, they have been devoid of virulence. On further inquiry amongst those who have harboured virulent bacilli they have elicited the fact that the latter were, in almost every case, recent contacts. After eliminating all such recent contacts it was found that virulent diphtheria bacilli occurred, and could not be satisfactorily accounted for, in two persons out of 1511. 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 (see Denny).

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

The results of the isolation of infected contacts[1].

The attempts which have already been made to stamp out diphtheria outbreaks by the examination of contacts, and the isolation of those found to be infected, have been attended with encouraging results.

[1] See note p. 259.

They have principally been made in isolated schools and institutions in the towns of Minnesota by the State Board of Health (1900), and by several observers in this country. More extensive outbreaks involving several schools in a town have been similarly treated also with a considerable measure of success by Cobbett (IV. 01, and X. 01) at Cambridge and Graham-Smith (1902) at Colchester.

Cobbett (XI. 1901) consequently considers that "the duty of discovering, isolating, and disinfecting the former class of persons (*i.e.* infected contacts) 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 call '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 to the immense advantage of the public health."

The measures adopted in this epidemic.

(1) Cultures were examined from all notified persons, and all those in whose throats diphtheria bacilli were found were isolated until three consecutive negative examinations showed them to be free from the bacilli. The majority were isolated in the infectious diseases hospital and the others at their own homes.

(2) As far as possible all children belonging to families in which cases of diphtheria had occurred, and all persons known, or likely, to have been in contact with the cases were also examined, and, if diphtheria bacilli were found in their throats, isolated. Particular attention was paid to the schools in this respect. Whenever a case occurred the class which the child attended, and on one occasion nearly the whole school, was examined. The parents of the infected children were informed of the fact, the dangers were explained to them, and in most cases their consent was readily obtained to the isolation of the children in an Isolation Home. In the few cases in which the parent's consent could not be obtained the children were kept at home, and not allowed to attend school. In all these cases three consecutive negative examinations were required before release from isolation.

(3) As far as possible all cases of sore throat brought to the notice of the medical practitioners, and more especially those occurring among school children, were investigated.

(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 well as the use of antiseptic mouth washes.

The work of carrying out all these administrative measures entirely rested with Dr Bushell Anningson, the Medical Officer of Health, and to him belongs the credit of the great measure of success which attended them.

General history of the epidemic.

The epidemic practically resolves itself into a series of outbreaks in various schools and institutions, most of which promptly ceased when the measures just mentioned were put in force.

The outbreak commenced in the middle of March 1903, when several scholars attending the Infants' Department of the St Matthew's School were notified to be suffering from diphtheria. After the examination of the school and the isolation of infected contacts, it was closed for other reasons, but some cases occurred amongst its scholars, and persons connected with them, probably by means of untraced contacts. In the Girls' Department two cases were notified about the same time, and examination revealed several healthy contacts. No further cases occurred in this department. In the Infants' Department, however, a recrudescence occurred in November, probably by means of an infected contact from the March outbreak. This child had been isolated at that time and released after three consecutive negative examinations. During the November outbreak he was again found amongst the infected contacts. On both occasions the bacilli isolated from this child were found to be virulent.

One of the first cases to be notified from the Infants' Department was isolated in the Hospital. By this means apparently several persons became infected. The type of the disease was, however, very mild, and most of the six notified persons had only slight clinical signs. This outbreak was promptly brought to an end by the examination of the patients in the infected ward and the attendants, and the isolation of those found to be harbouring the specific bacillus.

At the Sturton Street School one child was notified on March 24th.

This patient lived only a few doors from some of the infected contacts found in the St Matthew's School, and it seems likely she became infected from some of them.

The examination of the scholars only revealed one infected healthy child. In May two scholars of this school suffered from the disease, but it is extremely probable that they were infected in the same manner as the original case. No other cases occurred in this school.

At the Sanatorium a curious instance of the spread of the disease was noticed amongst Scarlet Fever cases[1]. One of these was found to have virulent diphtheria bacilli in a discharge from the ear. The examination of the other patients showed that five of them had diphtheria bacilli in their mouths. Three cases probably infected from this source were notified. After the discovery of the condition that existed, and the separation of the infected persons, no further cases occurred.

In November an outbreak appeared in the Catherine Street School, which was immediately suppressed by the examination of all the scholars and the isolation of the infected ones.

Besides the outbreaks just mentioned some isolated persons, in whom the source of infection could not be traced, were notified. Many of these only showed slight clinical signs, and were only certified on the finding of diphtheria bacilli. Further investigation in a number of the cases showed that the bacilli were non-virulent (pp. 270 and 284).

Detailed account of the results of the examinations of notified cases and contacts, and of the virulence of the diphtheria bacilli found.

St Matthew's School. (*Girls.*) The first case to be notified in which diphtheria bacilli were found was that of a child (1)[2] attending the Girls' Department of the St Matthew's School on Feb. 20th. Two examinations were made at this time but no diphtheria bacilli were found, but during the examination of the healthy children in this school in March, this child was found to be harbouring virulent diphtheria bacilli. It is of course possible that her illness was not

[1] For details see p. 268.

[2] The numbers refer to the tables at the end of the paper showing the period during which the bacilli were found in the mouth, the presence or absence of Hofmann's bacillus, the virulence of the diphtheria bacilli, and the results of the autopsies of the inoculated animals.

due to diphtheria bacilli, but to the streptococci which were found in large numbers, and that she subsequently became infected with diphtheria bacilli, but on the other hand it seems more likely from subsequent events that she was at first really infected with diphtheria bacilli, which were for some reason not discovered, and that her case was the starting point of the outbreak. Early in March another case was notified, but although examined on many occasions no diphtheria bacilli were found.

Towards the end of March two children (2, 3) developed the disease. The former showed virulent diphtheria bacilli, but the latter died before any examination could be made.

Within a few days 122 children from this school were examined and 7 with diphtheria bacilli found, including the child first mentioned. Six of these (1, 5, 6, 7, 8, 9) harboured virulent diphtheria bacilli, and one (4) non-virulent bacilli. With two exceptions (1, 9) all were treated at the Isolation Home.

No further cases have occurred in this department of the school.

St Matthew's School. (*Infants.*) Between March 10th and 14th four scholars (10, 11, 13, 14) attending this department of the school developed diphtheria, probably being infected from children attending the other department.

The bacilli obtained from three were virulent, but those from one (10) were not tested.

By March 22nd 279 children, scholars of the school and family contacts, had been examined, and 16 harbouring diphtheria bacilli discovered. Four of these (12, 15, 24, 29) subsequently developed the disease. The latter and eight others showed virulent bacilli (16, 17, 18, 19, 20, 22, 23, 25), and four (21, 26, 27, 28) non-virulent bacilli. All the healthy contacts harbouring virulent bacilli were treated at the Isolation Home.

Two other children (33, 34), associated with scholars of this school, were notified at this time. The former was a brother of one of the clinical cases (29), and the latter lived close to several of the infected children.

In May two scholars (30, 31) developed the disease. Both these as well as four other children (35, 36, 37, 38), who were notified in June, probably acquired the infection at their homes from some undiscovered source as all lived within a small area. One child (32), a sister of one of the notified scholars (31), was also found to be

harbouring diphtheria bacilli. The bacilli from all the nine children just mentioned were virulent.

The school, which had been closed from the middle of March, was opened early in June, but no further cases occurred for five months. Then a second outbreak occurred, probably caused by one of the infected contacts of the earlier outbreak. This child (67, No. 23 March outbreak) who had been found in March to be harbouring virulent diphtheria bacilli was allowed to go home after three consecutive negative examinations. In the outbreak which occurred in November he was again found to be harbouring virulent diphtheria bacilli.

In the November outbreak four scholars (61, 62, 63, 64) and two children (69, 70) connected with the school were notified to be suffering from diphtheria. All showed virulent bacilli. The class to which they belonged containing 59 children was examined, and four infected contacts (65, 66, 67, 68) were discovered. The bacilli isolated from all these were virulent. Two of these (66, 67) were treated at the Isolation Home.

Since that time no further cases have occurred.

Hospital. One of the first cases (14) to be notified at the St Matthew's School was treated at the Hospital. In March a case (45) occurred there, and no other source of infection could be found. Apparently infected through the attendant on this patient five cases occurred (46, 47, 48, 50, 49), all of a mild type, and only notified because diphtheria bacilli were discovered. Two of these were first discovered as apparently healthy contacts. The first four showed virulent, but the other had non-virulent bacilli. Amongst the patients in the infected ward one contact (51) with virulent bacilli was found.

This outbreak then ceased, and no further cases have occurred.

Sturton Street School. Towards the end of March a child (39) attending this school was notified to be suffering from diphtheria. The bacilli were virulent. Infection probably occurred at home through contact with one of the scholars of the St Matthew's School.

114 children from this school were examined, and one (40) found with virulent diphtheria bacilli.

In May two other scholars (41, 43) developed the disease, in all probability by contact with the children (35, 38) near whose homes they lived. The brother (44) of the latter was also attacked shortly afterwards. The bacilli discovered in these three children were virulent. Non-virulent diphtheria bacilli were also discovered in one (42) of the

family contacts of the first mentioned. Both the infected contacts found in connection with this school were treated in the Isolation Home.

No further cases have occurred in this school.

Sanatorium. Amongst the patients isolated here suffering from scarlet fever a curious outbreak occurred. In June a case (54) of diphtheria occurred and another patient (52) with a sore throat was notified on diphtheria bacilli being found in the culture. The bacilli in both these cases were virulent. Shortly afterwards it was discovered that one of the scarlet fever patients (53) with a discharge from the ear had virulent diphtheria bacilli in the pus. On this 49 persons who had been in contact with him were examined, and five (55, 56, 57, 58, 59) were found to have virulent diphtheria bacilli in their throats. On means being adopted to separate these persons the outbreak came to an end.

Catherine Street School. At the end of November two clinical cases (72, 73) of diphtheria were notified. Both showed virulent bacilli.

Five classes in this school were examined. In class iii of 54 children to which these patients belonged eight infected contacts were discovered. Seven of these (75, 76, 77, 78, 79, 80, 81) harboured virulent diphtheria bacilli. Infection may have been conveyed to this school through a patient (71) who suffered from the disease in May by means of one (77) of these infected contacts. The latter lived close to the former, and had been attending the school for some time with a sore throat. In any case the bacilli had probably been in this child's throat for some time, and the high percentage of infection can be accounted for in this way. It was noted that nearly all these infected children sat close together in school. One other child (74) also had diphtheria bacilli, but they were non-virulent.

In the fourth class with 54 scholars four (82, 83, 84, 85) infected with diphtheria bacilli were discovered. In the first two the bacilli were non-virulent, but in the latter two virulent.

In two classes (i and v) with 47 and 41 children respectively, none harbouring diphtheria bacilli were found, but in the second room with 102 children one (86) was found with virulent bacilli.

The classes chiefly infected were only separated by a curtain, and presumably mingled freely.

Of the contacts harbouring virulent diphtheria bacilli all but three (75, 77, 84) were treated at the Isolation Home. Three weeks later one further case (87) occurred and the outbreak then ceased.

Probably connected with this school through contact at home was the case of a girl (88) notified to be suffering from diphtheria early in January (1904). The bacilli isolated from this case were virulent, but those from her brother (89) were non-virulent. The patient worked in a dress-making establishment from which 30 persons were examined without finding diphtheria bacilli, and no cases of the disease occurred amongst them.

It is also possible that a patient (117) attending the *Ross Street School* was infected from the above source. 47 scholars belonging to the class which this child attended, and five family contacts were examined, but no diphtheria bacilli were found.

Post Office. In February 1904 one (95) of the employees in the Post Office was found to be suffering from a sore throat. On examination virulent diphtheria bacilli were found. Within a few days another similar case (96) occurred. Amongst the family contacts of the latter two (97, 98) harbouring diphtheria bacilli were discovered. In connection with these cases 52 persons connected with the Post Office, and 19 with a printing establishment, in which one of the contacts (97) worked, were examined, and one (99) healthy contact found. All the last four persons, who were almost certainly infected from the same source, were found to have non-virulent bacilli. (Also see Addendum, p. 321.)

New Street School. Early in March 1903 a scholar from this school died from a disease which appeared to be diphtheria. A thick membrane covered the palate and tonsils. Numerous cocci were found, but neither from the exterior, nor the interior of this membrane could diphtheria bacilli be cultivated, nor could they be demonstrated in sections made from it. Forty-three children from this school were examined, and one (91) with non-virulent diphtheria bacilli discovered.

Abbey School. About the same time a scholar attending this school was notified to be suffering from diphtheria, but no diphtheria bacilli were found on culture. Thirty-three children from the school were examined, and one (90) with non-virulent diphtheria bacilli discovered.

No cases of the disease occurred in these schools.

Two children (92, 93), who had been attending the *St Barnabas School,* suffered from sore throats and virulent diphtheria bacilli were isolated. A sister of these children also showed diphtheria bacilli on culture, but the bacilli could not be isolated. No other cases of the disease occurred in this school[1].

[1] Details of the occurrence of the pseudo-diphtheria bacillus amongst the scholars of these schools are given in Table VIII.

Certified cases of diphtheria in whom the source of infection was not discovered.

During the course of the year 91 suspicious cases of sore throats were examined, and in 12 of these diphtheria bacilli were found. The bacilli in seven of them were virulent (100, 101, 102, 103, 104, 105, 106). The source of infection in the first four was not definitely ascertained, but was probably connected with one of the schools. In the last three it is quite unknown, but in the first two of these the clinical signs were very slight.

Five persons (107, 112, 113, 114, 115) were also notified on very slight clinical grounds on the discovery of diphtheria bacilli in their throats. The organisms in all these cases were devoid of virulence.

In connection with the first of these, four (108, 109, 110, 111) family contacts harbouring diphtheria bacilli were found, in all of whom they were non-virulent.

It will be seen from the foregoing account that these efforts to check the disease met with a considerable degree of success.

In five out of the six institutions in which the measures were applied, immediate, and almost complete, success was the result. In the case of the Infants' Department of the St Matthew's School the results were not so encouraging. In the first outbreak the effect of the isolation of the contacts was to check the disease for a time, no cases occurring for six weeks. Then several cases occurred in the district from which most of the children are drawn. The subsequent outbreak in November was, however, immediately controlled.

The discovery of persons infected by contact at the homes of the scholars is a matter of considerable difficulty since infection has in almost all cases occurred before the patient or contact, through whom it has been conveyed, is discovered. Consequently only inquiries at the homes can determine how many persons were liable to have been recently infected, and these must be visited at their homes to obtain swabs. It is probable that infection was conveyed to the few notified persons, in whom its source was not discovered, by such unrecognized contacts.

Certain contacts during the first part of the epidemic were isolated in whose throats suspicious organisms were found. On further investigations most of these organisms were found to be Hofmann's pseudo-diphtheria bacilli, or bacilli differing from diphtheria bacilli in

culture though resembling them in morphology. No mention has been made of such persons in the foregoing account.

In the treatment of outbreaks of diphtheria the closing of schools has seldom been found to be effectual in checking the disease, and when the methods which have been used in this case are employed, the difficulties are increased by doing so. While the school remains open the occurrence of a fresh case can immediately be followed by the examination of the school contacts, and intimate friends of the patient, and the movements of the patients before the attack can be fairly easily ascertained. If the schools are closed on the other hand the children are at liberty during the day and play with each other, so that it becomes extremely difficult to trace and examine all the possible contacts.

Of the infected contacts found during the outbreaks 39 were completely isolated in the Isolation Home, or in some other way, but the consent of the parents to the isolation of 17 could not be obtained. Fortunately, however, 10 of these harboured only non-virulent bacilli, which appear to be non-pathogenic to man (p. 286).

Although, whenever possible, three consecutive negative examinations were required before release from isolation, in a few instances it was found impossible to enforce this rule. This was especially the case in those children who could not be efficiently isolated, and in whose throats the bacilli lingered for long periods.

From 13 persons (9, 31, 45, 48, 50, 65, 68, 77, 84, 93, 94, 102, 104) with virulent bacilli two consecutive negatives were, however, obtained, and from one person (113) with non-virulent bacilli. One negative was obtained from two persons (1, 53) with virulent, and from three (108, 110, 114) with non-virulent bacilli. Four persons (32, 52, 69, 92) with virulent bacilli, and five (26, 28, 90, 109, 112) with non-virulent bacilli still showed them on the last examination.

It is worthy of note that in 43 of the examinations for the release of persons harbouring diphtheria bacilli at the time when the organisms were scarce, they were only found on looking at the culture for the second time after twelve to twenty-four hours' further cultivation.

It has been previously mentioned that 91 cases of suspicious sore throat were examined, 79 without finding diphtheria bacilli, although at least fourteen of the latter were certified on clinical grounds to be suffering from diphtheria. Of these persons 44 were examined two or three times, nine on four occasions, and two on five. Several of these certified cases subsequently developed scarlet fever.

That a not inconsiderable number of persons notified to be suffering from diphtheria, especially during epidemics, show no bacteriological evidence of the disease is the experience of the majority of observers. Woodhead (1896), for example, found that 20 % of the certified cases out of 12,172 admitted to the Metropolitan Asylums Board Hospitals during the years 1895—6 showed no diphtheria bacilli on culture, and the Massachusetts Board of Health (1900) report that during five years of the 2461 cases diagnosed as diphtheria on clinical grounds 859 (35 %) were negative on bacteriological examination, whereas out of 2977 doubtful cases with insufficient clinical signs for diphtheria 824 (27 %) were positive.

The treatment of epidemics of any considerable size by the bacteriological method, especially if all the diphtheria bacilli and doubtful organisms are isolated, involves a considerable amount of labour. During this outbreak over 2200 cultures were examined, of which 757 were re-examined after further growth, and more than 350 organisms were isolated, of which 194 were fully investigated, namely 113 diphtheria bacilli, 26 pseudo-diphtheria (Hofmann's) bacilli, and 55 diphtheria-like organisms obtained from various sources.

In all, over 7000 cultures for diagnosis, and the observance of the characteristics of the bacteria in pure culture, were examined and the results recorded.

The characters of the diphtheria bacillus.

There can be little doubt "that when once one has become fully acquainted with the range of its variation it is fairly easy to recognize the diphtheria bacillus and distinguish it from all others," but "*the eye cannot be sufficiently trained for this purpose unless the observer frequently tests the opinions he forms on morphological grounds by isolating his cultures, and testing them in various ways, including the injection of animals*" (Cobbett, IV. 01, p. 236).

In the practical diagnosis of diphtheria it must be assumed that organisms morphologically resembling diphtheria bacilli are true diphtheria bacilli, for the opinion of the bacteriologist to be of any practical value cannot await the preparation of pure cultures and the injection of animals; but if deductions of any *scientific* value are to be drawn from the observations the testing of the organisms found is essential, especially if the deductions are to be made the bases on which epidemics of diphtheria are to be combated by bacteriological means.

Apparently in consequence of omitting to verify their diagnoses by isolating and testing doubtful organisms very divergent views are held even at the present day by various observers as to which of the various types of organisms should be considered dangerous. This unsatisfactory confusion renders many of the investigations untrustworthy, and can only lead to the discredit of the bacteriological diagnosis of diphtheria.

The divergent views held on this question are well illustrated by the Report of the Massachusetts Committee on "Diphtheria bacilli in well persons" (1902).

The several collaborators were requested to detail the various bacilli according to Wesbrook's types (1900), and also to state in each case whether or not a positive diagnosis of the presence of diphtheria bacilli had been made.

In Providence (Footnote, Report, p. 24) on the basis of the Committee's belief that *A*, *C* and *D* of Wesbrook's types should be considered chiefly, or solely, important, there would be ·43 % of positives (*i.e.* cultures in which diphtheria bacilli were present). If all granular, or barred forms, but not the solid forms, be included as Prof. Gorham of Providence states, there would be 3 % of positives. If all be included there would be 25 %. The number actually reported positive (*i.e.* diphtheria bacilli present) makes about 9 %.

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

It is evident from the above statement that some standard must be adopted in dealing with an epidemic. In order to prove the reliability, or otherwise, of the standard adopted, although it involved a considerable increase of work, I have isolated, cultivated on several media, and tested on animals 113 out of the 117 diphtheria bacilli discovered as well as several organisms derived from various sources resembling in morphology the diphtheria bacillus[1].

Hofmann's pseudo-diphtheria bacillus was also very frequently found and numerous pure cultures were made. The observations on this point are given later (page 297).

Morphology of the diphtheria bacillus.

Cobbett (IV. 01) has carefully worked out the virulence of the various types of bacilli met with during the outbreak of 1900 at

[1] For results see table at the end and descriptions of diphtheria-like organisms.

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 (1902) and in this epidemic.

He recognizes five morphological types of diphtheria bacilli on young serum cultures.

(1) Oval bacilli with one unstained septum. Very young forms[1].

(2) Long, faintly stained, irregularly beaded bacilli (Plate XIV, Fig. 1).

(3) Long regularly beaded bacilli—"streptococcal" forms (Plate XIV, Fig. 2).

(4) Segmented bacilli (Plate XIV, Figs. 3, 4, 5).

(5) Uniformly stained bacilli (Plate XIV, Fig. 6).

In all these types except the first the organisms are usually considerably, occasionally three or four times, longer (3—6 μ) than the pseudo-diphtheria bacillus. They are nearly always decidedly curved, of varying thickness, and often with decidedly clubbed ends. The arrangement of the bacilli in the field has been likened to Chinese characters, or pine needles on the ground.

The medium used throughout this and the other epidemics just mentioned was clear alkaline ox, or horse serum to which 1 % of glucose had been added, first prepared by Prof. Lorrain Smith of Belfast (1894).

The morphological appearances were those noted after 18—24 hours' growth on this medium at 37° C.

Several experiments were made to ascertain whether different alkalis (NaOH, KOH and Na_2CO_3) made any difference in the morphology, but none was noted. For routine purposes the alkali used was a 10 % solution of caustic soda, and it was generally found necessary to add about 1 c.c. to every 100 c.c. of the serum.

This medium has the advantage of being clear so that the differences of the colonies can be easily seen, also diphtheria and Hofmann's bacilli grow readily in 24 hours to the exclusion of most other organisms.

Comparative experiments between Löffler's medium and the above have convinced me that both organisms grow as readily on the latter as on the former, and that the morphology and cultural characteristics of both organisms are the same on these media. On the clear alkaline serum the inspection of the colonies is more readily accomplished, and

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

consequently it is easier to pick out suitable colonies for examination and isolation at an early stage. In this respect the alkaline serum is superior to Löffler's, and since in all other respects it is equally good, it has been used throughout for diagnostic work.

On one occasion ox serum could not be obtained and human pleuritic exudate was made use of. The result was that the Hofmann's bacillus became difficult to differentiate from the diphtheria bacillus, owing to large numbers of segmented forms being observed. In consequence a considerable number had to be subcultivated before a certain diagnosis could be given, but after subcultivation only typical Hofmann's bacilli were found.

Subsequent experiments with media made from human serum and pleuritic exudate showed that in every instance diphtheria bacilli became longer, more curved and segmented on these media as compared with the same organisms on alkalized ox, or Löffler's serum. The Hofmann's bacillus on human serum showed a tendency to become longer and develop segmentation, and on human pleuritic exudate was often definitely segmented, and curved. In fact many of the organisms closely resembled those shown in Plate XV, Fig. 7.

Media made from hydrocele fluid as recommended by White (1895) and Hayward (1895) were unsatisfactory.

The colonies of diphtheria and pseudo-diphtheria bacilli on alkalized serum after 24 hours' growth at 37° C. are the same in size and appearance, namely small, round, smooth, gray, and dome-shaped.

Methods of obtaining cultures and microscopical examination.

Swabs were prepared, constructed of cotton-wool wrapped round a short 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 on the surface of a serum tube. The culture so obtained was grown at 37° C. for 18—24 hours.

At the time of the examination samples from dissimilar colonies were streaked on cover-glasses by means of a sterile platinum needle, stained with diluted Löffler's methylene blue, mounted in the stain

and examined under $\frac{1}{12}$ oil immersion lens[1]. Unless the growth was very scanty, or the culture very thickly studded with colonies, organisms from more than one colony were never placed on the same position on the cover-glass. By this means the appearance of the various colonies, and the morphology of the bacilli derived from them could be studied, and the difficulties of distinguishing the organisms in a general smear avoided (see p. 316).

This method has very considerable advantages over the method generally adopted of staining with Löffler's methylene blue and finally mounting in Canada balsam. In the first place much time is saved, the bacilli are not overstained, nor are they liable to be distorted during manipulations or by the action of the balsam. Comparative experiments by the two methods showed that in balsam the diphtheria bacilli and others appeared smaller, and shrunken, and were much less easily distinguishable from other organisms than when treated by the method just described. Finally this procedure allows of 5 % acetic acid being run under the cover-glass as recommended by Cobbett (IX. 1901). This results in the organisms being partially decolourized, but the polar bodies in the case of diphtheria and other bacilli possessing them remain stained, and stand out as black dots in the faintly stained bacilli. A single group of doubtful organisms can thus be readily examined for the presence of polar bodies without removing the eye from the microscope, a matter of great importance when the organisms are few, and it is doubtful whether they can again be obtained for making a preparation by Neisser's method.

Comparative experiments with this method and that described by

[1] More in detail the process consists of making on a cover-glass several parallel streaks from different colonies. To accomplish this the top of a colony is touched with a platinum needle, the organisms separated by rubbing the point in water, and then drawing the needle across the cover-glass. By this means thin bands of well separated organisms from various colonies are left on the cover-glass. The cover-glass is allowed to dry and then dropped film side down on a glass slide on which has been placed a drop of dilute Löffler's methylene blue (1 : 5). The specimen is almost immediately firmly pressed down on filter-paper with the cover-glass downwards, with the result that the excess of stain is forced from under the cover-glass, and absorbed by the blotting-paper. Immersion oil is then placed on the cover-glass and the preparation examined mounted in a small quantity of the staining fluid. If the films are properly prepared no bubbles are found under the cover-glass, and the organisms take up the stain and appear to lie in a clear fluid (see Cobbett and Phillips, XII. 1896, p. 197). If segmented the segments are shown as dark blue bands and the presence of polar bodies is generally indicated.

Neisser show that the results are identical. By the adoption of these procedures the time required in making examinations is much shortened, and with experience over 100 cultures can be thoroughly examined by one observer in the course of the day.

Polar bodies.

Contrary to the experience of most observers a large number of the diphtheria bacilli found during this epidemic possessed in the original cultures few and small polar bodies or none. This was especially noted in the bacilli obtained from both notified persons and contacts infected with virulent bacilli from the St Matthew's School in both outbreaks and the Sturton Street School.

Most of these bacilli were of the type shown on Plate XIV, Fig. 3. This condition was not so common amongst the later cases when well-marked polar bodies were usually present.

Those bacilli which were originally without polar bodies usually did not show them in the first subcultures, but after continued growth on artificial media they made their appearance, and were present in fresh subcultures after 18 hours' growth.

The lack of polar bodies has been taken by some authorities to indicate a want of virulence, but in this epidemic polar bodies were more often absent in the virulent than in the non-virulent forms.

Of the 88 *virulent diphtheria bacilli discovered* 43·1 % *showed well-marked,* 30·7 % *small and poor, and* 26·1 % *no polar bodies; and of the* 25 *non-virulent diphtheria bacilli* 88 % *showed well-marked,* 8 % *small, and* 4 % *no polar bodies.*

Cultures of these organisms grown on Löffler's serum behaved in the same way.

Hofmann's pseudo-diphtheria bacillus had as a general rule in young cultures no polar bodies, but in a few instances inconspicuous polar bodies very few in number were seen. In subcultures from colonies of the latter the organisms were of the usual type without any signs of polar bodies. In older cultures small and indistinct polar bodies occurred in a small proportion of the organisms.

Most of the diphtheria-like organisms (pp. 301—312) possessed very distinct polar bodies.

The presence of polar bodies is therefore a considerable aid to diagnosis, but their absence in organisms morphologically resembling diphtheria bacilli does not prove that they are not virulent and dangerous diphtheria bacilli.

Cultural characters of the diphtheria bacillus.

All the diphtheria bacilli that were discovered in, and isolated from, 113 different persons were grown in glucose broth. All without exception formed acid in 48 hours, whereas no single specimen of the large number of Hofmann's pseudo-diphtheria bacilli which were grown on this medium did so.

The growth of the diphtheria bacilli in sugar-free, or glucose broth was in all instances as generally described, namely the fluid remained clear and a deposit of fine granules formed on the bottom and sides of the test-tube. The deposit was generally more marked in cultures in glucose broth.

This test for the production of acid, which, according to most observers, ranks next in importance to the inoculation of animals, although it serves to differentiate the diphtheria bacillus from the pseudo-diphtheria bacillus, does not distinguish the former from certain other diphtheria-like organisms (*pp.* 301—312).

All the diphtheria bacilli were also grown on slightly alkaline *potato*, and in most cases there was either no visible growth, or a very slight white glazed film. Certain of the diphtheria-like organisms however grew luxuriantly on this medium.

Numerous cultures on *gelatin* and *agar slopes* failed to show any constant well-marked differences between diphtheria and Hofmann's bacilli. It was found, however, that in *agar stab* cultures the diphtheria bacillus frequently only produced a scanty and thin growth on the surface, though in some instances it was fairly abundant. Hofmann's bacillus on the other hand grew extremely well on the surface, producing a white, rounded, smooth, luxurious mass occasionally marked by concentric rings.

In the examination of certain ear discharges and normal ears (p. 311) organisms resembling the diphtheria bacillus were found which grew well on potato, and it occurred to me that a differential medium might be made from potato juice stiffened by the addition of agar. This *potato-agar* medium was made, and it was found that diphtheria bacilli produced characteristic colonies, entirely different from those of any other diphtheroid organisms which have been tested.

This medium was prepared in the following manner. The pulp and fluid obtained by crushing washed, peeled, potatoes in a mincing machine was added to water in the proportion of one gramme of pulp to 1 c.c. of water. The mixture was allowed to stand in a flask for 12 hours and then was filtered through filter

paper. Agar after the usual treatment, to the extent of 3 %, was then added to the filtrate, and the flask was placed in a steam sterilizer at 100° C. till the agar was melted. After cooling the white of egg was added, and the flask again placed in the steam sterilizer till the fluid was clear. The medium was then filtered through a Chardin filter paper, filled into tubes, and sterilized on three occasions at 100° C. in steam. This is spoken of as *Acid Potato Agar*.

After standing, the mixture of pulp and water is decidedly acid. A second medium called *Alkaline Potato Agar* was made by neutralizing the acid with normal caustic soda, using litmus as the indicator, and adding 3 c.c. of alkali per litre before the addition of the agar.

The first of these media is clear, but slightly opalescent like ordinary agar, and the second is also clear but of a brownish colour.

As it occasionally happens that on tubes from one flask of a medium an organism produces a certain type of colony, but on tubes from other batches of the medium, though made in the same way, another type grows, both kinds of the potato-agar medium were made on four separate occasions at considerable intervals. Diphtheria and Hofmann's bacilli and most of the other organisms described later behaved in the same manner in all the samples of this medium whether acid or alkaline. Only two organisms differed markedly in their growth in the two varieties of this medium.

Although subsequent experiments may show that this medium is not so reliable as I have found it to be, yet it seems worthy of an extended trial. During this outbreak nearly all the 113 diphtheria bacilli, which have been isolated, as well as all the diphtheroid organisms (each several times) and nearly 100 pure cultures of Hofmann's bacillus have been grown on it, and no difficulty has yet arisen in differentiating by their growth after 48 hours the diphtheria bacilli from all the other organisms.

The colonies of the diphtheria bacillus on acid, or alkaline potato-agar are small and transparent, or gray, after 24 hours' growth at 37° C. After 48 hours, however, they have considerably increased in size. Two somewhat different forms occur.

(*a*) One is flat, gray, and round, with a small more opaque slightly raised mass in the centre, which has a rough granular surface. The rest of the colony has also a slightly granular surface, but the granulations are much finer (Plate XVII, Fig. 1). Very rarely such colonies have deep indentations and are star-shaped (Plate XVII, Fig. 2).

(*b*) The second type of colony is also round and flat, but is almost transparent. Again there is a small elevation in the centre, which in this case is very slightly granular, surrounded by an almost transparent

faintly granular zone, but the edge of the colony is raised in a decided rim as high as the central elevation (Plate XVII, Figs. 3 and 4).

Both these types of colony are more pronounced when the colonies are sparsely scattered. When the colonies are closely aggregated they never attain a size sufficient to show their characteristic features.

These two types of colony do not seem to be in any way connected with the virulence or want of it in the organisms. The great majority formed the type (*a*), and frequently these as the results of longer growth came to closely resemble type (*b*).

Hofmann's pseudo-diphtheria bacillus forms medium-sized, round, whitish, opaque, smooth, dome-shaped colonies entirely different from the above (like the colonies shown on Plate XVII, Fig. 5). All the other diphtheria-like organisms investigated with two exceptions form also colonies more or less of the dome-shaped type.

Consequently this medium, in one or other of its forms, seems to provide an additional and ready means of separating the diphtheria bacillus from other bacilli which resemble it in morphology, and Hofmann's bacillus.

Several specimens of the diphtheria and Hofmann's bacillus were tested for the formation of indol, but in most cases none was formed, or the quantity produced was very small, whereas some of the diphtheria-like organisms produced it in abundance.

According to Hewlett (1901) in the case of the diphtheria and of the Hofmann's bacillus the pink reaction obtained by the addition of strong acid to the weak nitrate solution is not due to indol, which is volatile, whereas this is due to a non-volatile substance, skatol-carbolyxic acid.

The virulence of the diphtheria bacillus.

The great majority of those who have investigated this subject have come to the conclusion that diphtheria bacilli show all degrees of virulence, from the highly virulent bacilli which in doses of ·1 c.c. of a 48 hours' broth culture injected subcutaneously kill half-grown guinea-pigs within three days to organisms completely lacking in virulence of which 2 c.c. produce no effect.

Abbott (1902, p. 400), for example, says, "under certain circumstances with which we are not acquainted *Bacillus diphtheriae* may become diminished in virulence, or may lose it entirely, so that it is no longer

capable of producing death of susceptible animals, and may cause only a transitory local reaction from which the animal entirely recovers.

"This exhibition of the extremes of its pathogenic properties, viz., death of the animal, on the one hand, and only very slight local effects on the other, was at one time thought to indicate the existence of two separate and distinct organisms that were alike in cultural and morphological peculiarities, but which differed in their disease-producing power. Further studies on this point have, however, shown that the genuine diphtheria bacillus may possess almost all grades of virulence."

In fact the prevailing opinion appears to be that under certain unknown conditions, or from lengthened stay in the throat, the virulent diphtheria bacillus gradually becomes attenuated until it may become completely non-virulent.

This opinion has been arrived at by the discovery of diphtheria bacilli, which only kill guinea-pigs after several days, and others which only cause local tumour without death. Councilman (1893, p. 548) for instance thinks that "like other organisms the diphtheria bacillus varies greatly in virulence," and Andrews (1900) considers that "short of fatal results the production of pathogenic effects, such as illness and local tumour in the inoculated animal is consonant with the belief that the bacillus is an attenuated form of the diphtheria bacillus."

At least two observers, however, have found that in their experience there are no intermediate degrees of virulence between the fully virulent and completely non-virulent organisms.

Theobald Smith and Walker (1896) in a series of most carefully conducted experiments on the toxin-producing power of 42 cultures of diphtheria bacilli from different sources found that all the organisms produced the same amount of toxin, or were of equal virulence. Eleven of these cultures were made from the throat 15—62 days after the disappearance of the disease. They did not meet with any non-virulent forms.

Spronck (1895, 1898) showed that the variations of toxin-producing power of diphtheria bacilli grown in broth were due to the presence of varying quantities of muscle sugar in the medium, and recommended a sugar-free broth for the production of toxin.

Theobald Smith (1896) also pointed out "that the amount of toxin decreased as the presence of muscle sugar grew larger, and that the best was obtainable only from beef nearly free from this substance."

In the majority of observations on the virulence of diphtheria bacilli no mention is made of the kind of broth in which the organisms

were grown, or the dose injected, and it is possible that the condition of the broth as regards muscle sugar may to some extent account for the various degrees of virulence which have been met with.

More recently Cobbett (x. 01, p. 496) says: "I do not deny that diphtheria bacilli may become attenuated, but think it interesting to note that in a somewhat extended experience partially attenuated bacilli have never been found. Fifty-five diphtheria cultures have been separated and tested for virulence during the spring, making with the 24 isolated and tested during the autumn and winter 79 in all." Moreover in seven cases he tested the virulence of the bacilli present on from two to ten occasions and found it constant.

My experience during this epidemic is in entire agreement with that of these authors. 113 cultures of organisms morphologically and culturally identical with diphtheria bacilli have been isolated and tested with the result that 87 have been found capable of killing half-grown guinea-pigs in three days, and 25 have been completely non-virulent. One for reasons to be given later, only killed in 12 days.

In order to render the conditions in these experiments on virulence as far as possible constant, almost all the bacilli were grown for 48 hours at 37° C. in muscle-sugar-free broth from the same stock. It was found that all did not grow in this medium equally well on the first inoculation. In cases in which the growth was poor a second tube was inoculated by transferring a loopful of broth from the first tube to a second. Under these conditions, when the organisms became accustomed to the medium, abundant growth was generally obtained.

Guinea-pigs weighing between 150 and 400 grammes were selected, and from ·1 to ·3 c.c. of the 48 hours' broth cultures was injected subcutaneously.

Under these circumstances, when the conditions had been made as constant as possible, 87 specimens of diphtheria bacilli killed within three days, or at the outside limit on the fourth day. Leaving some margin for the differences due to the inequalities of growth, the susceptibilities of the animals and their different sizes, all of these may be regarded as fully virulent bacilli.

The one specimen which only killed after 12 days could not be induced to grow well in broth, only a few granules resulting after 48 hours' incubation. Several transferences from one broth culture to another still resulted in very poor growth. Consequently the dose was much smaller than in the other cases, and it cannot be assumed that this was an attenuated form.

Cobbett (x. 01, p. 496) also, in two instances, found organisms which grew poorly in broth, and only killed after several days, one on the 11th and the other on the 7th day. In each case, however, when the injections were repeated with a new culture death took place in the usual time.

Autopsies were made on all the animals which died. In every case the well-known signs of death from experimental diphtheria were found, namely subcutaneous oedema, general congestion of the organs, especially marked in the supra-renal capsules, and in some cases fluid in the pleura.

At the site of injection a small grayish mass was always found from which the bacilli could be obtained. In all cases cover-glass preparations were made from this point, and the morphology of the bacilli noted. All the various morphological types of the bacilli, which have already been described as occurring in cultures, were found in these preparations[1]. The gelatinous oedema varied greatly in amount; from a comparatively small area round the site of injection to a condition in which the whole ventral subcutaneous tissue of the abdomen, thorax and thighs was involved. In most cases this oedematous mass was clear and gelatinous, but in a certain proportion of cases minute haemorrhages had occurred into its substance. The conditions have been described in the tables at the end as extensive, moderate, slight and haemorrhagic.

The degree of injection of the supra-renal capsules was also variable, ranging from dark red, almost black, to pink. The conditions met with have been described by the terms dark red, red, and pink in the tables.

In these autopsies the condition of the lungs was especially noted. They varied from deep red, mottled with darker areas, to pink or whitish.

As far as possible the quantity of fluid in the pleural cavities was ascertained. This varied from 12 c.c. to none. The fluid in all cases was clear, without any trace of blood, and no relationship seemed to exist between the quantity of fluid and the degree of injection of the lungs.

In most of the earlier experiments ·1 c.c. of a 48 hours' sugar-free broth culture was injected, but in some of the more recent ones up to ·3 c.c. was used. This was done in order to ascertain whether the larger doses within these limits caused death to take place earlier or produced more extensive lesions. The average time of death was, however, not altered, and no differences were discovered at the autopsy.

The injection of these small doses (·1—·3 c.c.) in the case of non-virulent

[1] Compare Ohlmacher, p. 290.

bacilli produced no results, or only a very transitory oedema lasting a few hours. In many of these cases other animals were injected with doses of 2 c.c. with the same results. Roux and Yersin (1890, p. 47) have pointed out that in some cultures they obtained colonies some of which were virulent and others non-virulent. To guard against the possibility of having separated non-virulent bacilli only from patients in whom virulent bacilli were also present, the second injection, whenever possible was made with bacilli isolated from another culture. Also the organisms were taken from several colonies in this culture. With these precautions against missing any virulent, or slightly virulent, bacilli, the results were always the same, viz. the inoculation of 2·0 c.c. gave rise to no tumour or signs of illness in the inoculated animal.

It will be noticed that in several (10) of the inoculation experiments given at the end of the table (p. 327) 2·0 c.c. of broth culture were injected on the first occasion. The cultures were derived from persons who were known to have been in contact with patients in whom non-virulent bacilli occurred. In every instance the bacilli were found to be non-virulent, though identical with diphtheria bacilli in other respects.

The larger dose was also used in the case of cultures derived from persons who were suffering from very mild sore throats, and in whom inquiry revealed no possible source of contact with persons harbouring virulent bacilli. In all these cases with one exception (116) the organisms were non-virulent.

So far as I am aware this is the only outbreak of any size in which the diphtheria bacilli, from almost every person found to harbour them, have been isolated and tested. Moreover in no other outbreak on so large a scale have the persons liable to be infected been so thoroughly investigated.

On glancing over the table it will immediately be seen that the proportion of non-virulent bacilli bears no relation to the number of virulent bacilli either in notified persons or contacts. Moreover in the first three schools in which large numbers of children were examined the percentages of non-virulent bacilli were alike (·9 %), whereas the percentage of virulent bacilli varied widely in both notified cases (4·3—·6 %) and infected contacts (4·1—1·6 %). In the other institutions small numbers of persons only were examined in each case, consequently the percentages obtained are not of much value, but when the proportions are taken on the total number of persons examined in the last nine, it is found that the percentage of non-virulent bacilli is nearly the same as in the first three schools.

Table IV.

The table gives the proportion of persons in, or connected with, infected, or non-infected, institutions in whom virulent, or non-virulent, diphtheria bacilli have been found.

Institution	No. of persons examined	Notified persons with diphtheria bacilli: Virulent		Notified persons with diphtheria bacilli: Non-virulent		Contacts infected with diphtheria bacilli: Virulent		Contacts infected with diphtheria bacilli: Non-virulent	
St Matthew's School									
Infants (March)	317	13* (4·1 %)		0		12 (3·7 %)		4 (1·2 %)	
,, (Nov.)	59	6 (10·2 %)	4·3 %	0		4 (6·7 %)	4·1 %	0	·9 %
Girls	132	3* (2·2 %)		0		5 (3·8 %)		1 (·7 %)	
Catherine Street School									
Class i	47	0		0		0		0	
ii	103	0		0		1 (·9 %)		0	
iii	64	2 (3·1 %)	·6 %	0		7 (10·9 %)	3·1 %	1 (1·5 %)	·9 %
iv	63	0		0		2 (3·1 %)		2 (3·1 %)	
v	41	0		0		0		0	
Sturton Street School	125	3	2·4 %	0		2	1·6 %	1	·8 %
Abbey School	33	0		0		0		1 (3 %)	
New Street School	43	0		0		0		1 (2·3 %)	
Park ,, ,,	20	1 (5 %)		0		0		0	
Ross ,, ,,	47	1 (2·1 %)		0		0		0	
Dress-making establishment	30	1 (3·3 %)	3·6 %	0	·5 %	0	1·9 %	0	1·3 %
Sanatorium	59	3 (5·1 %)		0		6 (10·2 %)		0	
Post Office	62	1 (1·6 %)		1 (1·6 %)		0		3 (4·8 %)	
Hospital	48	5 (10·4 %)		1 (2·1 %)		1 (2·1 %)		0	
Printing Office	19	0		0		0		0	
Total†	1409	54 (3·8 %)		7 (·56 %)		37‡ (2·6 %)		19‡ (1·3 %)	

The total percentages of the bacilli obtained from notified cases and contacts with virulent bacilli was 6·4 % and with non-virulent bacilli 1·8 %.

* The virulence of one specimen in each case not tested, but assumed to be virulent as the organisms derived from the other notified cases were so (10, 3).

† These include the persons just enumerated together with certain other notified persons and family contacts. Four persons first discovered as contacts and subsequently notified have been placed under the head of "Notified" in the totals only.

The records of two persons with virulent bacilli have been omitted by mistake from the tables at the end. They are recorded below. The significance of the symbols may be seen by reference to the tables at the end. Both at the time of writing were still isolated.

116 W.F. (a) Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.O.Δ.Δ.Δ.Δ.O.Δ (* * * * * *) 30 acid 245 2·0 MS + extensive dark red 1·5 c.c.

117 A.S. (a) Δ.Δ ? ,, 300 ·2 SS LS + ,, ,, -

‡ One person in each case added in whom the virulence not tested, but assumed in the one instance to be virulent and in the other non-virulent, as this was the case in two and four other members of their families tested (94, 109).

The proportion of non-virulent diphtheria bacilli in the whole number of persons examined was 1·8 %.

It is therefore evident that in this epidemic at any rate from 1 to 2 persons in every hundred, whatever the proportion infected with virulent bacilli, harboured non-virulent diphtheria bacilli in their throats. There is a remarkable coincidence between these figures and those obtained by the examination of 1500 normal persons (p. 262) amongst whom about the same number (2·05 %) showed non-virulent bacilli.

In three households five (107—111), four (96—99) and two (74 and 82) persons had non-virulent bacilli. It may be safely assumed that the remaining members were in each case infected from one person. If these eight persons be excluded there remain 18 (1·2 %) in 1401 who harboured non-virulent bacilli not derived so far as is known from other persons possessing them.

Two instances occurred in which one member of a family possessed virulent bacilli and a second member non-virulent, but in each case the member harbouring the virulent organisms had recently been in close contact with other persons in a similar condition.

Consequently in this epidemic there is no evidence to show that non-virulent diphtheria bacilli can give rise to virulent, but on the other hand the three families just mentioned demonstrate that non-virulent bacilli when transferred from one person to another still remain non-virulent. Moreover no clinical cases of diphtheria are known to have arisen from such persons although no less than 13 of them refused to be removed to the Isolation Home. It is also worthy of note that none of the notified cases from which non-virulent diphtheria bacilli were isolated showed any marked clinical signs. In fact without the discovery of the organisms the illness would in most cases have been passed over as a slight sore throat. In the cultures derived from the majority of these cases colonies of the diphtheria bacillus were rare, but colonies of other organisms, especially cocci, were abundant. It would seem likely therefore that the clinical symptoms were entirely due to the latter.

All these observations tend to show that diphtheria bacilli which are non-virulent for guinea-pigs are also non-pathogenic to man. If this conclusion be correct it may be questioned whether it is necessary to isolate these persons, and whether, if isolated amongst those who carry about the virulent diphtheria bacillus, they are not liable to catch diphtheria. In practice, however, the virulence of the bacillus can only be determined after isolation has been carried out, and

accordingly several children with non-virulent bacilli lived in the Isolation Home with others who had virulent bacilli. This, however, was followed by no bad results.

It should be noted in this connection that Cobbett (x. 1901, p. 491) found that those admitted to the Home with a non-virulent bacillus were never found to have acquired a virulent bacillus during their stay, nor was a non-virulent bacillus ever found in a child in whom virulent bacilli had once been found. In the case of one child the bacilli were isolated and tested 10 times in the course of 15 weeks and were always found to be non-virulent. During five weeks her sister was with her constantly, and on three occasions bacilli were isolated from her and found fully virulent. Moreover from another girl, who remained in the Home about as long, diphtheria bacilli were isolated and found fully virulent on no less than six occasions.

There is no evidence to prove that bad drains and insanitary environment can ever convert non-virulent into virulent bacilli, or originate diphtheria. Shattock (1898) experimented on this question and found that 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.

One cannot deny that the virulent diphtheria bacillus may become attenuated, *but it is interesting to note that in the two most extended observations on this subject, namely the two consecutive outbreaks investigated by Cobbett and this one, in which* 79 *and* 113 *specimens of diphtheria bacilli,* 192 *in all, have been isolated and tested for virulence, no partially attenuated diphtheria bacilli have been found.*

Taking into consideration the morphological and cultural resemblance of the Xerosis bacillus, frequently present in the normal eye, to the non-virulent diphtheria bacillus, the very close resemblance of certain organisms found in the mouth and ear, and the similar distribution of non-virulent diphtheria bacilli in contacts and non-contacts, it may be that the older view which regarded many of the latter as belonging to a distinct species may be more correct than the one at present generally accepted.

As will be shown later (pp. 301—312) some of the organisms obtained from the mouth and ear so closely resemble non-virulent diphtheria bacilli that they would be by many observers undoubtedly classed as such.

The persistence of diphtheria bacilli in the throat.

In several of the recorded outbreaks patients and infected contacts have been released from isolation after one negative examination. The need for more than one negative examination has, however, been clearly established. Hill (1898) states that the Boston Board of Health, U.S.A., require two consecutive negatives from convalescents, and three from hospital patients before they are declared free from infection.

At the South Western Fever Hospital, London, the patient is detained until the bacilli disappear as evidenced by three consecutive negative examinations. Cobbett (IV. 1901, IX. 1901) 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 bacilli.

In this outbreak the mean duration[1] in the throat of virulent diphtheria bacilli amongst notified persons in whom the disease was not fatal was 36 days, of non-virulent bacilli 15 days. Amongst contacts with virulent bacilli it was 30 days, and with non-virulent bacilli 25 days. The length of their stay in notified persons with virulent bacilli varied between 99 and 8 days, in notified persons with non-virulent bacilli between 51 and 1 days, in healthy contacts with virulent bacilli between 57 and 8 days, and in contacts with non-virulent bacilli between 122 and 8 days.

The following table gives the results of some of the investigations on this subject.

TABLE V.

Observer	Mean duration of persistence of diphtheria bacilli in the mouth	Longest period noted	Shortest period noted	
Park (1894)	8	49	3	(from the disappearance of the exudate)
Morse	10	—	—	
Bissel (V. 1902)	14	—	—	
Cobbett (IV. 1901)	18	49	3	(3 consecutive negatives required)
,, (X. 1901)	—	108	—	
Massachusetts Board of Health (1901)	27	185	2	(for a period of 5 years)
Graham-Smith (1902)	28	94	—	(3 consecutive negatives required)
Woodhead (1896)	52	200	—	
Wesbrook (1900)	—	135	—	

Most of these observers require less than three consecutive negative examinations for release.

[1] The duration of persistence is reckoned from the date on which the bacilli were first found to the date of the first of the three consecutive negative examinations.

Out of the 104 convalescent patients carefully examined at Colchester (Graham-Smith, IV. 1902) 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 examination, on three after two consecutive negatives and once after four.

In this outbreak a single negative followed by the reappearance of the diphtheria bacilli often for a long period occurred on 49 occasions, two consecutive negatives on 22 occasions, three on two occasions, and six on one.

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

Wolff (1895) found diphtheria bacilli in fatal cases in the frontal and ethmoidal sinuses, and the antrum, and Councilman, Mallory and Pearce (1901) in the antrum and middle ear.

A glance at the tables and the figures just given shows 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[1] have been obtained, and to insist on more would be impossible. Therefore, I think, whenever practicable, a minimum of three consecutive negatives should be enforced before convalescent cases, or infected contacts, are freed from isolation.

Hofmann's pseudo-diphtheria bacillus.

The pseudo-diphtheria, or Hofmann's, bacillus is an organism very frequently met with in the throats and noses of the scholars at the public schools.

Much diversity of opinion has existed and to some extent still exists, as to the relationship between this organism and the diphtheria bacillus. On the one hand there are some who consider that it is merely an attenuated form of the true diphtheria bacillus, capable under favourable conditions of developing into the latter, and giving rise to diphtheria, others appear to think that only certain species included under this

[1] See observations on this point p. 271.

name are attenuated diphtheria bacilli, whilst others again consider that this organism is in no way related to the diphtheria bacillus, and is at all times perfectly innocuous to man.

The question of the relationship, if any, which exists between these two bacilli is not a matter of scientific interest alone, for the entire management of an outbreak from the bacteriological standpoint depends on the view which is taken. Hewlett (1899, p. 203) for example, believing "that Hofmann's pseudo-diphtheria bacillus is a modified diphtheria bacillus," goes so far as to say that a positive diagnosis of diphtheria should be given whenever it is found.

If the opinion of those who consider Hofmann's bacillus to be merely an attenuated variety of the diphtheria bacillus be correct, measures such as have been described, and which in several outbreaks have proved so efficacious, ought to be of little use, since (according to this view) organisms capable under certain unknown conditions of giving rise to diphtheria remain in the throats and noses of nearly half the school children. Had this view been adopted, about 500 school children in Cambridge would have had to be isolated in order to render the measures efficacious.

These observers base their opinions on certain experiments, and on the statement that the pseudo-diphtheria bacillus is found more frequently amongst convalescents from diphtheria, and persons who have been in contact with cases of the disease, than amongst normal persons.

Some of the experimental evidence in favour of this view is as follows:

Roux and Yersin (1890, p. 418) were able to attenuate virulent diphtheria bacilli by growing them at a high temperature. Although they were able to increase the virulence of lowly virulent bacilli, they were unable to do so with completely non-virulent forms (p. 423).

Hewlett and Knight (1897) considered that on one or two occasions they succeeded in transforming a pseudo-diphtheria into a virulent form, but have been apparently unable to repeat these transformations. Richmond and Salter (1898) briefly stated that by repeated passages through certain birds they had been able to convert Hofmann's into diphtheria bacilli virulent for guinea-pigs, and Salter (1899) in the following year gave details of some of these experiments.

Ohlmacher (1902) experimented with three organisms, and concluded that by a short sojourn in an immune animal a diphtheria bacillus may be converted into a pseudo-diphtheria, and that the reverse may be

brought about by passing the organism through a susceptible animal. His experiments only show, however, that a long granular diphtheria bacillus, after recovery from the subcutaneous tissues 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 passage through an immune animal became granular and non-virulent.

Lesieur (1901) in agglutination experiments on the two organisms concluded that certain species of the pseudo, but not others, were identical with diphtheria bacilli. On the other hand Lubowski, working with the serum of animals immunized to a non-virulent diphtheria bacillus, found that it agglutinated not only these bacilli, but 23 quite typical races of diphtheria bacilli. The serum had no action on pseudo-diphtheria bacilli.

The above are some of the main experimental arguments in favour of the identity of the pseudo-diphtheria and diphtheria bacillus, "but before accepting the conclusion that *B. Hofmanni* is convertible into *B. diphtheriae*, and on this account a factor in the causation and spread of diphtheria, more evidence is necessary, particularly with regard to the strict purity of the cultures used, and also with regard to the number of cases in which such a conversion may be considered to have occurred. Caution is particularly necessary when it is called to mind that the evidence at one time advanced in support of the conversion of *B. anthracis* into *B. subtilis*, and at another of *B. coli communis* into *B. typhosus*, has in both cases been discredited" (Gordon, 1901, p. 420).

In support of the view that the pseudo-bacillus is an attenuated form of the diphtheria bacillus, it has been repeatedly stated that it occurs more frequently in the throats of those who are recovering from an attack of diphtheria at the time when the true diphtheria bacillus is disappearing. It must be remembered, however, that in diagnostic work, when once the diphtheria bacillus has been found no further search is usually made, but that in subsequent examinations, when the diphtheria bacilli are becoming less numerous, a more careful examination is necessary, and the pseudo-bacillus is found and recorded.

During this epidemic Hofmann's pseudo-diphtheria bacillus has been considered to be entirely unrelated to the diphtheria bacillus and innocuous to man, but though no importance has been attached to its presence it has been recorded whenever it has been found.

Twenty-three cultures of this bacillus derived from persons suffering

from diphtheria, or infected with diphtheria bacilli, were isolated and tested on animals to ascertain whether they showed any signs of virulence. Six of these were isolated from the first cultures, and three from the second from notified persons (all with virulent diphtheria bacilli), eight from infected contacts at the first examination (seven with virulent and one with non-virulent diphtheria bacilli), and seven from contacts at the second examination (seven with virulent and one with non-virulent diphtheria bacilli)[1]. If this organism is an attenuated form of the diphtheria bacillus it would seem probable that under these circumstances, when the true diphtheria bacillus was still present in very large numbers, its virulence would not have been completely lost. *All, however, behaved in the manner typical of the pseudo-diphtheria bacillus, giving an alkaline reaction in glucose broth, and being completely devoid of virulence for guinea-pigs in doses of* 2 *c.c. of* 48 *hours' broth cultures.*

Further, it will be seen by reference to Table VIII, p. 297, and to the more detailed tables at the end of the paper, that Hofmann's bacillus was not found more frequently in the throats of convalescents and healthy infected contacts than in those of normal persons, although the former were all examined on many occasions. In most cases it was either present or absent throughout the series of cultures examined from each person, and did not appear to be in any way related to the diphtheria bacillus.

The characters of the pseudo-diphtheria, or Hofmann's, bacillus.

The organism which in these observations is called the pseudo-diphtheria, or Hofmann's, bacillus has the following characteristics.

In young serum cultures it appears as a darkly staining oval bacillus of somewhat variable length, with one narrow unstained septum. Occasionally colonies are met with, which contain a fair number of bacilli with several septa. Young subcultures from these colonies, however, usually show only the typical oval forms. It frequently happens also that besides the well-stained forms oval unstained specimens of the same size and shape are found. There is a marked tendency for these organisms to be arranged in small groups, several members of which lie

[1] Notified persons from whom subcultures of Hofmann's bacillus were obtained at the first examination were 11, 12, 24, 39, 54, 88, at the second examination 33, 100, 116. Infected contacts were 7, 8, 22, 32, 65, 75, 81, 99 at the first, and 4, 6, 16, 17, 23, 25, 79 at the second examination.

parallel to one another. No polar bodies usually occur in young cultures, though occasionally colonies are found in which some of the organisms have very small indistinct polar bodies.

Besides these typical oval forms, I have, both on previous occasions and in this epidemic, 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, produce an alkaline reaction in glucose broth, and are non-pathogenic to guinea-pigs. 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 become visible. This is termed the pseudo-diphtheria type of Hofmann's bacillus (Plate XV, Figs. 7 and 8)[1]. This type has generally been found in the noses of persons suffering from catarrh, and occasionally in the mouth. The altered conditions under these circumstances may account for the difference in morphology[2].

The colonies of the typical and atypical forms of the Hofmann's bacillus on serum are indistinguishable from those of diphtheria.

Subcultures were made from colonies of Hofmann's bacillus containing some, often a considerable number, of long segmented bacilli from nearly 100 persons. In every case in subculture the typical form of the bacillus alone, or combined with a few long forms, was seen, and all that were tested produced an alkaline reaction in glucose broth.

The different reactions produced in glucose broth by the growth of diphtheria and Hofmann's bacilli have already been compared (p. 278), and the cultural differences on potato-agar fully detailed, the former producing colonies of one of the two types recorded and the latter round, smooth, dome-shaped colonies without distinctive features.

Cobbett (IV. 1901, p. 243), in a former outbreak in this town, isolated and tested for virulence 69 specimens of this bacillus, derived from persons recovering from the disease, contacts and normal people, and all without exception were completely devoid of virulence in doses of 2 c.c. injected subcutaneously and produced an alkaline reaction in glucose broth. All examples which I have previously tested have

[1] Graham-Smith (1903, p. 230).

[2] This organism differs from the *Bacillus coryzae segmentosus* described by Cautley (1896) and which has been also found by myself in similar circumstances (p. 302).

behaved in a similar manner. In consequence of these observations I have only thought it necessary to test for virulence specimens obtained from persons suffering from the disease in early stages, infected contacts, and cases of suspicious sore throat in which no other diphtheria-like organisms had been found. The negative results of these experiments have already been given (p. 292). More than 150 examples of the pseudo-diphtheria bacillus were, however, also isolated from convalescents, contacts and normal persons, and grown on various media. All without exception produced an alkaline reaction in glucose broth, and behaved in other respects after the characteristic manner of these bacilli.

The investigations of nearly all observers show that both amongst contacts and normal children attending public schools the percentage of infection with the pseudo-diphtheria bacillus is very high, owing to the fact that sweets, slates, pencils, etc., pass from one child to another, and to the habit children have of placing their fingers, and such articles as pencils in their mouths. The absence of such habits amongst older persons may account for the smaller percentage of infection amongst them with this bacillus.

During the outbreak at Colchester (1901) I examined 563 cultures from contacts of all classes not infected with the diphtheria bacillus, and found the bacillus of Hofmann on 316 occasions (55·4 %). 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 the percentage of cases in which it occurred was much lower. In schools attended by the children of the poor where many articles were shared in common, and want of strict attention to cleanliness was frequently observed, 64·5 % harboured this bacillus.

In the following table it will be noticed that there was no relationship between the percentage of persons infected with Hofmann's bacillus and that infected with the diphtheria bacillus. Almost all the persons mentioned in the second half of the table were adults of the well-to-do class who had not been in contact with diphtheria. The percentage of infection with the pseudo-diphtheria bacillus amongst them was small (19 %), for the reasons which have been explained, and is only slightly less than that (22·7 %) found in well-to-do persons at Colchester amongst whom a large number were infected with the diphtheria bacillus.

TABLE VI.

The table gives the percentage occurrence of Hofmann's bacillus amongst school children and others at Colchester, and amongst healthy non-contacts, the majority belonging to the well-to-do class, at Cambridge.

Class of persons examined	No. of cultures	Percentage infected with Hofmann's bacillus	No. of healthy persons infected with diphtheria bacilli*	
Colchester				
Scholars. School I	6	66·6	0	These schools were attended by the children of the poor 64·5 % infected with Hofmann's bacillus
,, II	30	66·6	0	
,, III	50	64·0	8	
,, IV	49	63·3	8	
,, V	149	63·0	6	
,, VI	59	62·7	5	
,, VII	16	62·0	1	
,, VIII	15	60·0	5	
,, IX	37	57·0	5	
,, X	9	33·3	1	Better class schools 31·5 % infected with Hofmann's bacillus
,, XI	10	30·0	0	
Persons above school age	40	50·0	3	
Well-to-do persons	79	22·7	23	
Cambridge				
Scholars. School XII	29	0	0	Better class schools
,, XIII	49	12·2	1	
Patients in Addenbrooke's Hospital	98	12·2	1	Persons above school age 19 % infected with Hofmann's bacillus
Undergraduates of Sidney Sussex College	41	21·9	3	
Members of the University	48	4·1	0	
Workers in the Pathological Laboratory	18	22·2	0	
Other persons	198	24·7	0	
Total	1044	36·4	70	

* Not included in the numbers of examinations.

The next table shows that the percentage infection with Hofmann's bacillus is very variable, according to these observers ranging from 78·9 to 5·7 %. It again, however, demonstrates that the proportion of persons infected with Hofmann's bacillus bears no relation to the proportion infected with the diphtheria bacillus. Taking the school children alone it is seen that nearly one-third harbour the pseudo-diphtheria bacillus in their mouths.

TABLE VII.

The results of observations by various investigators on the occurrence of Hofmann's bacillus in healthy persons.

Observer	No. of persons examined	No. of times Hofmann's bacillus found	Percentage infected with Hofmann's bacillus		Percentage infected with the diphtheria bacillus	Remarks
Cobbett (1903)	1495	536	35·8		?	Mostly school children
	19	15	78·9	39·4	5·0	Scholars, children of the poorer class, attending three schools
	49	37	75·5		0	
	120	76	63·3		6·6	
Minnesota Board of Health (1901)	30	21	70·0		3·3	Mankato School, p. 533
	50	30	60·0		6·0	Faribault ,, p. 526 (feeble minded)
	40	22	55·0		5·0	Owatonna ,, p. 531
	57	22	38·6	31·6	10·5	Faribault ,, p. 529 (public schools)
	193	52	25·9		13·7	Owatonna ,, p. 519
	242	31	13·2		28·5	Bethany Home, p. 535
	24	2	8·3		25·0	Albert Lea Schools, p. 522
Chatin & Lesieur (1900)	75	22	29·3		2·66	School
Hewlett & Murray (1901)	385	92	23·9		15	Children admitted to a General Hospital
Berry & Washbourn (1900)	142	33	23·2		12	Schools
Goadby (1900)	586	99	16·9		31·4	,,
W. Pakes (1900)	3000	446	14·8		14·3	Sore throats
Park & Beebe (1)	330	27	8·2		0	Healthy persons, see p. 262
Herman Biggs	330	19	5·7		10	,, ,, ,, p. 261
Total	7233	1624	22·4			
	(3265	1059	32·1)			School children

From the following table (VIII) it will be seen that the infection with Hofmann's bacillus amongst the scholars of the public schools in this outbreak was very high, ranging from 72 to 35 %. It is particularly interesting to note that the school which showed the greatest infection with the diphtheria bacillus showed the lowest infection with Hofmann's bacillus, and that schools in which no virulent diphtheria bacilli were found showed a very high degree of infection with Hofmann's bacillus. *This table again demonstrates that there is no relation between the numbers infected with virulent diphtheria bacilli and those infected with pseudo-diphtheria bacilli.* This point is made especially clear in the case of the Catherine Street School where the percentage of infection with Hofmann's bacillus in all five classes is practically identical, whereas in only three were diphtheria bacilli, virulent or non-virulent, found.

TABLE VIII.

Showing the proportion of persons infected with pseudo-diphtheria bacilli and with virulent and non-virulent diphtheria bacilli in the various schools and institutions examined during this outbreak.

Schools	No. of persons examined	No. in whom Hofmann's bacillus found	Percentage infected with Hofmann's bacillus		Percentage infected with virulent diphtheria bacilli*		Percentage infected with non-virulent diphtheria bacilli*		
Sturton Street School	120	72	60·0		4·1		·8		
Ross ,, ,,	46	28	60·0		2·1		0		
Abbey School	32	19	59·3		0		3·0		
New Street School	42	22	52·3		0		2·3		
Catherine Street School									
Class i	47	25 (53·1)			0		0		
,, ii	102	50 (49·0)			1·0		0		
,, iii	54	28 (51·8)	51·1		14·0	3·7	1·5	·9	
,, iv	59	27 (45·7)			3·1		3·1		
,, v	41	26 (63·4)			0		0		
Park Street School	20	10	50		0		0		
St Matthew's School									
(March)	288	105 (36·4)			5·5		1·2		
(Autumn)	49	24 (48·9)	37·4		14·0	6·6	0	1·02	
Girls	122	43 (35·2)			6·1		·7		
	1022	479	46·8		4·8		1·07		
Dress-making establishment	29	7	24·1		3·0		0		The majority of these persons were adults
Sanatorium	49	10	20·4		15·5		0		
Post Office	52	8	15·3		1·8		1·8		
Hospital	40	6	15·0		17·5		2·2		
Printing Office	19	2	10·5		0		0		
	189	33	17·4		8·5		·9		
Notified persons with diphtheria bacilli	52†	25	48·0	50·0	88·7		11·3		
Infected contacts	52†	27	51·9		67·2		32·7		
Suspicious cases	79	13	15·4		—		—		
Total	1409	577	40·9		6·4		1·8		

* These persons have not been included in the totals given under each head, but are given separately near the end of the table.

† Only those persons have been included here in whom two or more examinations have been made. In the totals, however, all persons examined have been included.

Further it is again shown that amongst adults a much smaller proportion harbour the pseudo-diphtheria bacillus in their throats, only 17·4 % showing them as against 46·8 % of the children, although 8·5 % of the former were infected with the virulent diphtheria bacillus as against 4·8 % of the latter.

A point of still greater interest in view of the statements which have been made is the proportion of persons infected with Hofmann's bacillus amongst the notified cases and contacts harbouring the diphtheria bacillus. In these two classes 48 % and 51·90 % respectively showed at some period pseudo-diphtheria bacilli in cultures from their throats.

Most of these persons were children attending the public schools, and it is seen that the total proportion infected (50 %) *with the pseudo-diphtheria bacillus almost coincides with the proportion infected with this organism amongst the healthy scholars attending the same schools* (46·7 %). These figures are all the more striking when it is remembered that each of these persons was examined on many occasions and the opportunities for finding the pseudo-diphtheria bacilli if they existed in their throats were very great. The results of each examination in these two classes are given in the tables at the end, and an inspection of these makes it clear that in some cases the pseudo-diphtheria bacilli were found throughout, whereas in other cases they were never found.

From these observations I am of the opinion that this bacillus is not more frequently found amongst the latter class of persons than amongst normal persons, and that it does not replace the diphtheria bacillus when that organism is disappearing, but that it is either present or absent in the cases under examination throughout the whole period[1].

"In view of the wide distribution of Hofmann's bacillus amongst healthy persons in Cambridge and elsewhere the conclusion arrived at by Richmond and Salter that the pseudo-diphtheria bacillus is a 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 established and it has been found capable of being converted into the virulent diphtheria bacillus, not only by laboratory procedures but further under natural conditions, we must not conclude that the causal agent of diphtheria is widespread" (Cobbett, IV. 01, p. 247).

[1] It must be remembered that at the time when some of these examinations were made the pressure of work was very great, consequently time could only occasionally be spared to look for the pseudo-diphtheria bacillus once the diphtheria bacillus had been found in the culture. Hence the omission to state that the former organism was present when the latter was found cannot be taken to mean that it was not present in the culture under examination. In cases, however, in which several examinations had been made without finding the Hofmann's bacillus special care was taken to thoroughly examine subsequent cultures for its presence.

These observations, I think, confirm the statements I have already made in regard to this organism (1903, p. 250), namely:

(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 adults, both of the poorer and well-to-do classes, and even amongst the children of the latter class.

(4) That it is probably spread 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 (1903, pp. 238—241).

(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 morphologically resembling diphtheria bacilli on serum cultures.

(*a*) *From the nose and throat.*

Apart from Hofmann's bacillus which only presents difficulties in diagnosis when giant forms, or the pseudo-diphtheria type alone, are present, various diphtheroid organisms have been described from the throat and nose.

Attention here has only been paid to those which grow on serum, and which bear a close resemblance to the diphtheria bacillus, when cultivated on that medium[1].

Cautley (1896) has described an organism which he has named the *Bacillus coryzae segmentosus*, obtained from the nasal secretion of seven out of eight cases of "Influenza Cold." Gordon (1901) has also isolated this organism, and has supplemented Cautley's account of it.

On *serum* after 18 hours' growth at 37° C. the colonies are very small. The organisms are of medium length and segmented. Some clubbed forms are present. They retain Gram's stain, and show polar bodies by Neisser's method. On *gelatin* at 20° C. small round grayish-white colonies are produced, and on *agar*

[1] The cultural characters in which these organisms markedly differ from diphtheria bacilli are in italics.

at 37° C. transparent colonies, which become grayish-white, and eventually filmy at the edges. *Broth* remains clear and growth chiefly takes place in small flakes forming a *scanty filmy deposit.* On all these media the bacilli are short and oval. In *dextrose broth* a *feeble acid reaction* is produced, and the same is the case in litmus milk, but clotting does not occur. It is non-pathogenic to guinea-pigs.

Cautley also isolated an organism from the mouth, only differing from the above in producing a flocculent deposit in *broth*, which becomes granular on shaking; and another from the nose differing in producing a thick white growth on *agar*, and turbidity and much white deposit in *broth.* This author appears not to have grown his bacilli on potato or glucose broth, nor to have made animal inoculations.

Gordon (1901) also isolated four other species of diphtheria-like bacilli.

No. 6. Grows well on *serum* in 18 hours at 37° C., but the organisms are much smaller than diphtheria bacilli, though they are segmented and clubbed. They retain Gram's stain, and show polar bodies by Neisser's method. On *agar* there is a good raised white growth, but the bacilli have no polar bodies. There is no growth on *gelatin. Broth* becomes slightly turbid and *conglomerate flocculi* are formed at the bottom of the tube. In *dextrose broth no acid* is produced. *Litmus milk* becomes alkaline and no clotting occurs. It is non-pathogenic to guinea-pigs.

Nos. 7 and 8. On *serum* produce *coherent growth*, but give rise to an acid reaction in *glucose broth.* No. 7 shows polar bodies by Neisser's method, but No. 8 does not.

No. 9 was isolated on four occasions from the throats of persons suffering from diphtheria.

On *serum* in 18 hours growth is moist, gray and raised, but not copious. Great variations occur in the shape of the organisms, every gradation between oval and bloated pear-shaped forms being found. They retain Gram's stain, and show polar bodies by Neisser's method. On *agar* round, gray, raised colonies develop which *adhere* to the medium. The colonies on *gelatin* are gray and in 10 days the medium begins to be *liquefied. Broth* shows a slight turbidity and a *weedy sediment.* Acid is formed in *dextrose broth. Litmus milk* becomes acid, and is *clotted* on the sixth day. It is non-pathogenic to guinea-pigs.

Excellent illustrations accompany the papers of these two authors.

Abbott (1891) discovered in the mouth an organism indistinguishable from the non-pathogenic diphtheria bacillus except in the fact that it formed a *dirty brown layer on potato.*

Davis (1898) isolated from the mouth in certain cases of scarlet fever a short diphtheria-like non-motile bacillus which in doses of 2 c.c. subcutaneously produced a general *septicaemia* and peritonitis in guinea-pigs. On *agar* it produced colonies with *dark centres and uneven edges,* and an acid reaction in *glucose broth.* A. Williams (1898) also seems to have isolated the same species from a patient who had suffered from diphtheria six weeks before.

Ruediger (1903) has recently described an organism, which he calls a virulent pseudo-diphtheria bacillus.

Seven strains of this organism were isolated from seven fatal cases of scarlet fever with gangrenous tonsilitis. Diphtheria antitoxin had no influence on the patients, and in fact in two cases its injection appeared to hasten death. The bacillus is described as resembling the true diphtheria bacillus in morphology, causing "*uniform turbidity* in *broth*, a soft, moist, and whitish growth on *agar*, a *hardy light brown* growth on *potato*, and turning *litmus milk white* in 5—6 days." Apparently all strains showed polar bodies by Neisser's method, and were agglutinated in dilutions of 1 in 200 by the serum of a rabbit which had received three injections of 24 hour broth cultures of strain No. 5. "Guinea-pigs are *not protected* against this organism *by anti-diphtheria serum*. All 7 strains are pathogenic for guinea-pigs after having been kept on agar for several months, when injected intraperitoneally in doses of 4 to 5 c.c." At the autopsies "the serous cavities contained a moderate quantity of fluid. The liver, spleen, and kidneys were markedly congested. The organisms could be isolated from the peritoneal cavity, heart's blood, and internal organs." A protective serum for guinea-pigs against injections of living cultures was obtained from a rabbit.

The author makes no mention of the reaction of his cultures in sugar media and appears to use the term pseudo-diphtheria bacillus as equivalent to the non-virulent diphtheria bacillus.

Diphtheria-like organisms isolated from the mouth and nose during this outbreak.

Owing to the confusion produced by the method of simply describing micro-organisms without the application of a scientific name, I have decided even at the risk of the possible creation of synonymic titles to give names to the bacilli, whose characters are described in the following pages, choosing ones which indicate some marked peculiarity in morphology or growth, or the source from which the organisms were obtained.

Note. In the following descriptions the cultural characteristics in which the organisms decidedly differ from the diphtheria bacillus are marked by italics.

Some of these organisms have now been cultivated on artificial media for nearly a year. All have been grown on several (6—8) occasions on each of the media on which their characteristics are noted.

Owing to the fact that the precise measurements of bacteria are but of little use in diagnostic work, the terms "long," "short" and "of medium length" are used to describe the bacilli. The term "bacilli of medium length" indicates organisms about the length of the Hofmann's bacillus (*i.e.* about 1·5 μ) and the term "short," organisms considerably shorter. The term "long" is used of organisms about the usual length of the diphtheria bacillus (3—6 μ) or longer.

By "large" colonies are meant colonies over ·15 cm. in diameter, by "medium-sized" ones about ·1 cm., and by "small" colonies considerably under ·1 cm. in diameter. The colonies are described as seen under a $5\frac{1}{2}\times$ Steinheil lens.

Unless otherwise stated the growth in sugar-free and glucose broth was similar.

In testing the reaction of glucose broth after 48 hours' growth at 37° C., the indicator used was neutral litmus.

Throughout these descriptions the term "non-pathogenic" means that 2 c.c. of 48 hour cultures in sugar-free broth injected subcutaneously were without effect on half-grown guinea-pigs during the period they were under observation (14—21 days).

All media with the exception of gelatin were cultivated at 37° C., the latter at 22° C.

All these organisms were tested for their power of reducing nitrates. Unless mentioned no reduction was found after 10—20 days' growth.

Spore formation has not been observed in any of these organisms.

1. *Bacillus coryzae segmentosus* Cautley.

Origin. Obtained from the nasal secretions of three persons suffering from coryza.

On *serum* grows more slowly than the diphtheria bacillus, though the colonies are very similar in appearance. *Organisms* in 24 hours resemble pseudo-diphtheria bacilli with light median bands, but are longer. A considerable number of long forms resembling uniformly stained diphtheria bacilli (Plate XIV, Fig. 6) are also found. They are non-motile, retain Gram's stain, and show small terminal polar bodies in most specimens. In *subcultures* many long segmented forms are seen, with two to four well stained segments, as well as shorter Hofmann-like forms. On *agar* in 48 hours *round, smooth, white, raised, dome-shaped colonies* are formed. The organisms are long, well segmented, curved and clubbed, and but few short forms occur. Polar bodies are only found in a few. They resemble closely the diphtheria bacillus shown in Plate XIV, Fig. 5. In *agar stab* cultures in 24 hours a small white round smooth surface growth is formed, which is very extensive after 48 hours. There is well marked growth in the depth. On *alkaline potato-agar* in 24 hours very small *round, smooth, gray, dome-shaped colonies* occur, which in 48 hours are *large, smooth, white and well raised above the surface* (like Plate XVII, Fig. 5). On *acid potato-agar a similar growth occurs.* On *gelatin* at 22° C. small round white colonies are produced. On *potato* an almost invisible thin whitish growth is formed. *Broth* remains clear, and *a stringy white deposit* is found at the bottom of the tube. *Glucose broth* in 48 hours is *very faintly* acid. Non-pathogenic.

The examples of this organism which I have isolated and tested agree in their characters with the descriptions given by Cautley and Gordon for the *B. coryzae segmentosus.* They differ from diphtheria bacilli in their rate of growth on serum, the characters of their growth on agar, potato-agar and broth, and in the degree of acid formation in glucose broth. In morphology the majority of specimens resemble the Hofmann's bacillus rather than the diphtheria bacillus, but the resemblance to the latter is striking in some of the larger forms.

2. *Bacillus diphtheroides citreus.* (Plate XV, Fig. 9.)

Origin. Obtained from the throats of five healthy children, two attending infected, and three non-infected, schools.

On *serum* in 24 hours the colonies closely resemble those of the diphtheria bacillus, but are rather larger and more opaque. After three days' growth they become slightly yellowish. The *organisms* are fairly long and stain darkly, in shape resembling Hofmann's bacilli, but with little trace of a median band. The majority are slightly curved, and show small terminal polar bodies, though in some of the longer forms they are very large and distinct. Non-motile, and retain Gram's stain. In 48 hour subcultures a number of long forms with three or four well marked segments and distinct polar bodies occur. On *agar* in 24 hours *large, white, round, smooth, moist, dome-shaped colonies* are formed. The organisms are the same in appearance as on serum, but the polar bodies are better marked. In *agar stab* cultures a flat, thick, moist, white surface growth with indented edges is formed. The indentations are very evident after 48 hours' growth. Along the needle track the growth is well marked. On *alkaline potato-agar large, round, white, smooth, dome-shaped colonies* are formed (like Plate XVII, Fig. 5). On *acid potato-agar* the growth is similar. *No growth* was obtained on *gelatin.* On *potato* in 24 hours a *very extensive pale yellow* moist growth occurs. The organisms are mostly short, but a number of markedly clubbed and segmented (up to 6 or 8 segments) forms are found. All show large and distinct polar bodies. *Broth* after 48 hours is clear with *white rather stringy deposit.* In *glucose broth* the deposit is copious, and white in *large flocculent masses* which tend to stick to the sides. The reaction is very acid. Non-pathogenic.

This organism differs slightly from the diphtheria bacillus in morphology though somewhat resembling Type 5 (Plate XIV, Fig. 6). It also differs in its growth on agar, potato-agar, broth and potato. It is from its marked growth on the latter that the name has been given. It resembles the organism next described very closely except in its growth on gelatin, potato, broth and glucose broth. It may be the same organism as that described by Abbott (1891).

3. *Bacillus diphtheroides brevis.* (Plate XV, Fig. 10.)

Origin. Obtained from a large abscess cavity opening into the mouth.

The colonies on *serum* in 24 hours are small, smooth, and white, but not so well raised as those of the diphtheria bacillus. Subsequently they develop somewhat filmy edges. *The organisms* in shape resemble Hofmann's bacilli, but are slightly curved and clubbed and show segmentation. The segments stain darkly, but the intervening bands except the middle one are not very definitely marked. They are non-motile, retain Gram's stain, and show polar bodies by Neisser's method. In subsequent subcultures segmentation is a marked feature. On *agar* stroke cultures

an *abundant, white, soft, slimy* growth is produced in 24 hours. The organisms are long, well curved, clubbed and segmented, and show good polar bodies resembling the diphtheria bacillus shown in Plate XIV, Fig. 3. *Agar stab* cultures show abundant white slimy surface growth which frequently becomes after further growth coarsely granular. A confluent, abundant growth occurs along the needle track with numerous projecting colonies. On *alkaline potato-agar* in 24 hours *large, round, smooth, gray, dome-shaped* colonies are formed, which in 48 hours are white and considerably larger (like Plate XVII, Fig. 5). The organisms vary from short oval to long well segmented forms. On *acid potato-agar* the colonies are similar. On *gelatin* in three days medium-sized, round, smooth, dry-looking colonies with raised centres develop. On *gelatin stab* cultures a large, white, dry, granular surface growth is formed, and small round colonies develop in the needle track. On *potato* in 24 hours an *extensive growth, thick, soft, and cream-coloured* is formed which gradually becomes granular and slightly *yellowish*. The organisms vary in appearance, some are short, but many long and well segmented with good polar bodies. In 48 hours *broth* becomes slightly cloudy and there is a fine whitish granular deposit. The reaction of *glucose broth* is extremely acid. *Milk* becomes *partially coagulated* in a few days and *indol* is *produced*. It is non-pathogenic.

This organism differs from the diphtheria bacillus in its growth on agar, potato-agar and potato, and somewhat in its morphology.

4. *Bacillus maculatus.* (Plate XV, Fig. 11.)

Origin. One colony found in a culture from the throat of a possible contact.

On *serum* in 24 hours the colonies are opaque white, but otherwise resemble those of the diphtheria bacillus. The organisms are longer and broader than diphtheria bacilli, but some short forms occur. The sides in some have slight bulgings at intervals. Numerous darkly stained segments cross the bacillus transversely in most of the organisms, but in a few there are oval segments. In some bacilli, especially in the later subcultures, long unstained intervals are seen. The organisms are non-motile, and retain Gram's stain very deeply. Each bacillus shows *numerous polar bodies* by Neisser's method; some of these are large and round, *others elongated transversely* across the bacillus, whilst others are *very minute*. These minute polar bodies are often very densely aggregated. The name indicates the remarkable spotted appearance seen when the organisms are stained by Neisser's method.

In the first culture the organisms lay in tangled masses of 10—50 individuals. On *agar* in 24 hours minute, round, transparent colonies are formed which subsequently grow very slowly. The organisms are about one-third the length of those found on serum cultures and of various shapes, from oval to bloated pear-shaped bodies. The polar bodies are few. On the surface of *agar stab* cultures an almost transparent film is formed, but in the depth medium-sized yellowish colonies grow. On *alkaline potato-agar* rounded, small, rather flat, slightly granular colonies with irregular edges are produced (Plate XVII, Fig. 6). The growth on *acid potato-agar* is similar. On *gelatin* after 10 days' growth the colonies are so *minute* as to be scarcely visible with a lens. Exceedingly

minute colonies also form in the depth of *gelatin stab cultures*. On *potato* there is no visible growth. *Broth* remains clear, but a *few very large, discrete, yellow granules* (0·5 *cm. in diameter*) are seen after 48 hours. The reaction of *glucose broth* becomes *very faintly* acid. *Milk* remains unchanged and no *indol* is formed. The organisms are non-pathogenic when injected either subcutaneously or intraperitoneally.

This organism differs from the diphtheria bacillus slightly in its morphology, its growth on potato-agar and gelatin, and in the very large size of the granules formed in broth, and the degree of acid formation in glucose broth.

5. *Bacillus diphtheroides liquefaciens.* (Plate XV, Fig. 12.)

Origin. Found in considerable numbers in the mouth of a patient suspected to be suffering from diphtheria.

On *serum* minute rounded colonies are formed in 24 hours, but in 48 hours they are medium-sized, round, slightly yellowish, dome-shaped and opaque. After 10 days' growth the colonies have sunk into slight pits, and the medium become *partially liquefied* after being kept 20—30 days at room-temperature. The *organisms* are very long, and markedly curved, and lie in groups more or less parallel to one another. There is very little clubbing, and but slight signs of segmentation, but all show well marked terminal and other polar bodies. Some specimens remain as unstained shadows. They are *motile*, but the movements are slow, and they retain Gram's stain. These organisms bear a fairly close resemblance to the diphtheria bacilli shown in Plate XIV, Fig. 5. On *agar stroke* cultures in 24 hours *a thick, moist, smooth, slightly yellow abundant growth* is formed. The appearance of the organisms is the same as on serum. On the surface of *agar stab* cultures an extensive moist smooth growth occurs, which occasionally in old cultures shows concentric markings. In the depth the colonies run together to form a continuous growth, the discrete colonies at the edges are rounded, but have blunt projections. On *alkaline potato-agar* in 48 hours round *smooth, moist, dome-shaped, slightly yellowish colonies* (like Plate XVII, Fig. 5) are formed, and the same is the case on *acid potato-agar*. On *gelatin* very minute, almost transparent colonies are formed. The medium becomes *liquefied* round them in about 10 days. In a few more days liquefaction is complete with a whitish-yellow mass lying in clear fluid. On *gelatin stabs* in 3 days the small yellowish surface growth is lying in a small cup-shaped area of liquefaction. In 11 days there is a *deep funnel-shaped hollow* with yellowish growth at the bottom and very minute colonies along the lower part of the needle track. On *potato* in 3 days a *thin extensive white growth* is formed, which in 6 days is *very abundant and yellow*. The organisms are of medium length, markedly curved, thin and stain uniformly. Many are clubbed and the polar bodies are very minute. *Broth* in 48 hours is slightly cloudy, with a large deposit of finely granular matter. In 48 hours the reaction of *glucose broth* is *neutral or faintly alkaline*. In 6—8 days *litmus milk* is *decolourized* and *firmly clotted*. No gas is produced, but much *indol* is formed and *nitrates reduced*. It is non-pathogenic.

This organism closely resembles some forms of the diphtheria bacillus, but differs in its growth on agar, potato-agar and potato, in the liquefaction of serum and gelatin, its reaction in glucose broth and its action on milk.

(*b*) *From the conjunctiva.*

Virulent diphtheria bacilli have been isolated from cases of diphtheria of the conjunctiva by Gordon (1) (1901), Jessop (3) (1895 and 1902), Eyre (1) (1897) and others.

An organism resembling the diphtheria bacillus very closely in many respects, known as the Xerosis bacillus, has been obtained from the conjunctival sac, in health and disease, by many observers.

Kuschbert and Neisser (1884) found the xerosis bacillus in large numbers in xerosis conjunctivae, Eyre (1897) in 12 persons with conjunctivitis, and many others have also found it in diseased conditions. Opinions however differ as to whether it is to be found in the healthy eye. Eyre (1897) examined 25 normal eyes, but could not find it, Stephenson (1898) out of 6209 normal children found it in 1·87 %, whereas Uthoft (1893) states that the xerosis bacillus is frequent in normal eyes and Lawson (1899) found it in 74·2 % of 200 persons examined (in 90 in pure culture). The latter observer considers it to be the most common and most universal inhabitant of the conjunctival sac.

Opinions also differ as to whether the xerosis bacillus is a non-virulent diphtheria bacillus, or a distinct species. Fraenkel (1896) inclined to the former view, but most of the other observers just quoted to the latter.

Eyre (1897) states that in the original cultures colonies only appear on *serum* after 48 hours, but in subsequent cultures grow well. The colonies are described as opaque, slightly heaped up, scaly, and adhering to the medium; and the organisms as resembling diphtheria bacilli in shape and segmentation. They are said not to differ in their growth on agar and gelatin from the diphtheria bacilli. *Broth* after 60 hours' growth remains *alkaline*. The organisms are non-pathogenic, and animals cannot be protected against the diphtheria bacillus by the injection of cultures of this organism.

Stephenson (1898, p. 63) was unable to produce xerosis in healthy human eyes by the inoculation of pure cultures of the xerosis bacillus. This author describes the organisms as retaining Gram's stain very tenaciously (p. 58).

Gordon (1901, p. 425) isolated an organism (No. 3) from a case of conjunctivitis which on *serum* in 48 hours formed a copious growth of *lemon-yellow* colonies. It resembled the diphtheria bacillus in morphology, retained Gram's stain, and showed

polar bodies by Neisser's method. On *agar* there was a copious growth with a *dry surface, and yellow pigmentation.* Growth on *gelatin* was like that formed by the diphtheria bacillus but *yellow. Broth* remained clear, and conglomerate yellow crumbs were found at the bottom. In *dextrose broth* an acid reaction was produced. It was non-pathogenic.

In the course of the observations I have examined by culture the conjunctivae of 10 healthy persons, and found the xerosis bacillus in 9. The culture in which it was not found was overgrown with a film-forming organism. Its cultural and morphological characteristics are as follows.

6. *Bacillus xerosis.* (Plate XVI, Fig. 13.)

Colonies only appeared on *serum* in the original cultures after 48 hours' growth and were then very minute. In subsequent cultures, however, the growth is more rapid. The colonies except in size resemble those of the diphtheria bacillus, but tend to *adhere* to the medium, and become irregular at their edges after 72 hours' growth. The *organisms* are of medium length, to long, and are curved, slightly clubbed and segmented. Forms with two to four segments are the commonest. In the original cultures polar bodies are rare, but in subcultures a few specimens show large polar bodies. The bacilli are frequently arranged in small groups. They are non-motile, and retain Gram's stain. On *agar* minute, raised, round, smooth, almost transparent colonies make their appearance in 48 hours. After days of growth very little increase in size takes place. The organisms are broader than on serum, well segmented, but show no polar bodies. In *agar stabs* the surface growth is small and almost transparent, and very minute colonies are seen along the line of puncture. On *alkaline potato-agar small, round, smooth, dome-shaped, gray* colonies, resembling those formed by Hofmann's bacillus, appear in 48 hours (like Plate XVII, Fig. 5). The majority of the organisms are short and like Hofmann's bacillus but many irregular forms are seen. On *acid potato-agar* the colonies are similar, but smaller. On *gelatin* only a few of the specimens showed any growth, the colonies being very minute, round, and transparent. In *gelatin stab cultures* only one specimen showed any growth along the needle track, and then only four very minute colonies appeared. On *potato* no visible growth occurred. *Broth* after 48 hours' growth remains clear and a few large granules are found at the bottom. In *glucose broth* the reaction of the cultures after 48 hours' growth varied slightly, being either *very faintly* acid, or neutral. *Milk* remains unchanged. It is non-pathogenic.

This organism resembles the shorter, segmented forms of the diphtheria bacillus very closely, but differs from it in its growth on serum, and potato-agar, in its very poor growth on gelatin, and in its power of producing acid in glucose broth.

As the xerosis bacillus appears to be a common inhabitant of the normal human conjunctiva, cultures were made from the eyes of a few

animals to ascertain whether similar organisms are to be found there. The eyes of 3 dogs, 3 rabbits, and 17 guinea-pigs were examined and organisms closely resembling the xerosis bacillus were found in the eyes of the dogs and guinea-pigs, but not in the eyes of the rabbits.

7. *Bacillus xerosis canis.* (Plate XVI, Fig. 14.)

Origin from the conjunctival sacs of the three dogs examined.

On *serum* the colonies only make their appearance after 2—3 days' growth, though in later subcultures the growth is a little more rapid. After 4—5 days' growth the colonies are of large size. Except for their size the colonies are indistinguishable in appearance from those of the diphtheria bacillus, but *tend to adhere* to the medium. The *organisms* are long, curved, and stain well, showing well differentiated, short, dark segments, separated by narrow light bands crossing the bacillus transversely. Clubbing in some specimens is well marked. These organisms resemble closely the pseudo-diphtheria type of Hofmann's bacillus (Plate XV, Fig. 7). By Neisser's method *variously shaped polar bodies* are seen *in large numbers* in each bacillus. Some are large and round, others elongated transversely across the bacillus, and many are exceedingly small. The organisms are non-motile, and stain well by Gram's method. On *agar* after 2—3 days' growth small grayish colonies with irregular edges, and darker centres appear. On *agar stab* cultures the surface growth is small and almost transparent, but in the depth a few medium-sized round colonies develop. On *alkaline potato-agar* after 2—3 days' growth medium-sized, *whitish, opaque, dome-shaped colonies, with a granular surface and very irregular margins* are seen. The organisms are oval and broad, but show well-marked segments. On *acid potato-agar* the growth is similar. On *gelatin no growth* was obtained. No visible growth occurs in *potato*. *Broth* after 48 hours remains clear but small granules are found at the bottom of the tube, which when shaken up *float in lines* as if held in position by invisible threads. The growth in *glucose broth* is similar, and its reaction after 48 hours is *neutral*. It is non-pathogenic.

This organism differs only slightly from the diphtheria bacillus in morphology but differs from it in its rate of growth on serum and its growth on gelatin, potato-agar and broth, and its reaction in glucose broth. It resembles closely in many respects the xerosis bacillus from the human eye.

14 (82 %) out of 17 guinea-pigs' eyes examined showed similar organisms. They resemble the organism just described in morphology, staining characteristics, and in their growth on all media except serum. On serum their colonies are the same in most cultures, but in some cases larger colonies develop which have a raised centre and raised rim resembling the second type of colony formed by the diphtheria bacillus on potato-agar (Plate XVII, Figs. 3 and 4).

These observations indicate that organisms closely resembling the diphtheria bacillus in morphology, and in many respects in cultural

peculiarities, but totally unconnected with them, are common inhabitants of the human conjunctival sac, and that of some animals.

(c) *From the ear.*

Virulent diphtheria bacilli have been isolated from the ear by some observers in a few instances, amongst others by Stevens and Parfitt (1897), and Gordon (1901, p. 424), and others have found organisms morphologically resembling the diphtheria bacillus. Pearce (1898) found such organisms in the middle ear in 25 out of 32 fatal cases of diphtheria. This observer remarks that "it is of interest that many of the cases showed no clinical evidences of ear trouble." Councilman (1893) found at the autopsy organisms which he considered to be diphtheria bacilli in acute inflammatory conditions of the middle ear in one case of diphtheria and two of measles, though the latter had not had diphtheria of the throat. Wright (2) also twice found these organisms under similar circumstances.

Egerton Williams (1901) found organisms which he regards as attenuated diphtheria bacilli in the ears of four children at a fever hospital. In one culture the rods were thick and frequently curved, and in the others short and of various thicknesses. They all formed acid in glucose broth, and were non-pathogenic to guinea-pigs. In conclusion (p. 1803) he says "that when organisms are found at all resembling the diphtheria bacillus they must in the present state of our knowledge be regarded as a modified variety of that organism, bearing in mind that their staining properties are often the only means of diagnosis available, the clinical symptoms being in this class of case often absent, and that these discharges (otorrhoea and rhinorrhoea) unassociated with sore throat, or symptoms, and therefore easily overlooked, may be the cause of unaccountable outbreaks, and the persistence of the disease amongst school children."

Duncan Forbes (1903) amongst 40 cultures from the ears of patients with scarlet fever found in 32 bacilli morphologically indistinguishable from diphtheria bacilli. Most of the organisms were of medium length; in a few cases however long bacilli were found; in some they were quite short, but had the characteristic appearance and staining properties of diphtheria bacilli. This observer does not state that he has made any cultural or virulence tests, but nevertheless regards all these organisms as diphtheria bacilli. Finally he regards it as possible that "if diph-

theria bacilli are present in the air of wards they may readily be carried by air currents."

Welch (1894), however, 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. Hill (1902) recently made some investigations on this subject and confirms Welch's conclusions.

Gordon (1901, p. 424) has also isolated a diphtheria-like organism from the ear of a scarlet fever patient.

> It resembles the diphtheria bacillus in morphology on *serum*, retains Gram's stain, and shows polar bodies by Neisser's method. On *agar* in 24 hours *opaque*, *white*, *homogeneous*, *glistening*, *and raised* growth occurs. On *gelatin* the growth is more uniformly consistent throughout, and *more coherent* than that of the diphtheria bacillus. It is non-pathogenic.

It has already been mentioned that in this outbreak a virulent diphtheria bacillus was obtained from the ear discharge of a scarlet fever patient (53, p. 268). This circumstance, together with the fact that diphtheroid organisms indistinguishable from the diphtheria bacillus in morphology were isolated at the same time from other cases, and the improbability of the conclusions at which Williams and Forbes have arrived as to the frequency of the presence of the diphtheria bacillus in the ears of such patients, led me to make some observations on the bacteriology of the ear.

The discharges from the ears of 10 scarlet fever patients were examined by culture, some on several occasions. One harboured virulent diphtheria bacilli, but three had organisms, described below as the *Bacillus auris*, resembling diphtheria bacilli, and three organisms to be described as the *Bacillus ceruminis*, resembling diphtheria bacilli but common in normal ears. Consequently 70 % had organisms morphologically resembling diphtheria bacilli, but differing in other respects.

Twenty normal ears of persons working in the laboratory were also examined by means of swabs, and 13 (65 %) had organisms more or less closely resembling diphtheria bacilli (*B. ceruminis*). Of all the ears examined, therefore, 66·6 % contained diphtheroid organisms.

8. *Bacillus auris.* (Plate XVI, Fig. 15.)

Origin. From the ear discharges of three scarlet fever patients.

On *serum* the colonies closely resemble those of the diphtheria bacillus, but grow more slowly. After 48 hours' growth the colonies are medium-sized. After 30 hours' growth the organisms are of various sizes, the majority of over medium length with darkly staining ends. They stain well, showing well marked segments with intervening light bands. In the longer forms several segments occur. Nearly all, even the shorter forms, are well curved, and have a tendency to be clubbed. A few pear-shaped forms were met with. They are non-motile, retain the stain by Gram's method, and show several well marked polar bodies by Neisser's stain. In sub-cultures the resemblance to diphtheria bacilli is still more marked. Nearly all the specimens are long, well segmented, and show several good polar bodies. The general arrangement in the field is similar to that of the diphtheria bacillus. On *agar* slopes in 24 hours the colonies are small, round, gray and dome-shaped. The organisms are short, curved and clubbed with well marked polar bodies. *Agar stab* cultures in 24 hours show a smooth, moist, white surface growth, and a good growth of discrete round colonies along the line of puncture. On *alkaline potato-agar* in 24 hours *smooth, gray, round, dome-shaped colonies* appear, which later become large and white (like Plate XVII, Fig. 5). The organisms are long, clubbed, and segmented. On *acid potato-agar* the colonies are smaller, but similar. On *gelatin* small, almost transparent, round colonies are formed in 48 hours, which in 3 to 4 days become *large and white, with a smooth surface slightly elevated in the centre.* The growth is *sticky and tenacious.* The organisms are the same as on serum. On *gelatin stabs* in 3 days there is an irregular, granular, white surface growth faintly marked by concentric rings, and good growth in the depth. On *potato* in 24 hours there is a *slight brownish-yellow growth, which in* 48 *hours becomes extensive, soft, yellow and glistening.* The organisms are short and oval, and stain well with large terminal polar bodies. *Broth* in 48 hours is slightly cloudy with a *white stringy deposit.* In *glucose broth* a copious, finely granular deposit occurs, and the reaction is very markedly acid. *Milk* remains unchanged. *Indol* is formed. The organism is non-pathogenic.

These organisms closely resemble the diphtheria bacillus in morphology and staining characteristics, but differ, more especially, in their growth on potato-agar, gelatin, potato, and broth.

9. *Bacillus ceruminis.* (Plate XVI, Fig. 16.)

Origin. 13 specimens were obtained from normal ears, and 3 from the ears of scarlet fever patients. 11 of these were fully investigated. The organisms appeared to be more plentiful when ceruminous secretion was present on the swabs. From this circumstance the name has been given.

On *serum* in 24 hours the growth is scarcely visible, but in 48 hours small round colonies, indistinguishable from those of the diphtheria bacillus, are formed. After 72 hours the colonies are medium-sized to large. Two forms slightly different in

morphology were noticed. (*a*) In 30 hours long thin and curved, and uniformly stained, with small terminal polar bodies. In 72 hours the bacilli are longer, more curved, well segmented, and with well marked polar bodies. (*b*) Medium length, slightly curved, uniformly stained, but markedly clubbed, with large polar bodies in a few specimens. After 72 hours the appearances of the two forms are similar. These organisms are non-motile, and retain the stain by Gram's method.

On *agar* after 24 hours the colonies are small, round, gray and dome-shaped, but later become large and white. The organisms are of medium length, curved, often clubbed, fairly well segmented, and show good polar bodies. On *agar stabs* a small, white moist surface growth is present in 24 hours, which after 48 hours' growth is often lightly marked with concentric rings. A confluent growth takes place in the line of puncture.

On *alkaline potato-agar* in 24 hours *medium-sized, opaque white, smooth, dome-shaped colonies appear.* On *acid potato-agar* the growth is similar, but more copious. No growth was obtained on *gelatin.* On *potato* in 24 hours *a whitish to yellowish white growth occurs, later becoming abundant and yellow.* After about 10 days the growth has a dry granular appearance. The organisms are mostly short, slightly curved, and clubbed. *Broth* remains clear and a small *stringy, white deposit* is formed. In *glucose broth* the deposit is granular and the reaction *neutral or alkaline. Milk* remains unchanged and no indol is formed. They are non-pathogenic.

In morphology and staining characters these organisms closely resemble the diphtheria bacilli shown in Plate XIV, Fig. 6, but differ in the rate of their growth on serum, their characters on agar, potato-agar, potato, and broth, and in producing no acid in glucose broth and no growth on gelatin.

The results of these observations show that a species of non-pathogenic organism, almost indistinguishable from the diphtheria bacillus in morphology, is present in the majority of normal ears, and that another species also resembling the diphtheria bacillus is frequently present in the ear discharges of scarlet fever patients. Under these circumstances it is essential to test thoroughly any organisms isolated from such situations before giving a diagnosis. The hasty generalizations made by two recent observers, Williams and Forbes, as to the frequency of the diphtheria bacillus in the ears of scarlet fever patients are probably based on the finding of the organisms which have just been described. The description of the organisms by the former, as far as it goes, and their lack of virulence correspond with these bacilli. The latter, however, appears to have entirely depended on morphological appearances. Evidence is, therefore, yet lacking that the diphtheria bacillus occurs in the ears of scarlet fever, or even diphtheria, patients with or without symptoms, except in a few instances.

(*d*) *Diphtheria-like bacilli from birds.*

"Diphtheria of the lower animals, especially fowls, and pigeons, has been made the subject of numerous investigations; and when we examine the literature we are immediately struck with the difference of opinion regarding the disease. On the one side we have those who believe that the one disease in man and birds is identical; and on the other side those who believe that the one disease has no relation to the other" (Harrison, 1903).

Harrison (1903) gives a short summary of the works of various writers and investigators, grouped under two heads:

(1) Those who have investigated the disease as it occurs in fowls and pigeons, by the usual methods employed in working out infectious diseases.

(2) Those who have made observations without experimental research, and who did not employ bacteriological methods to support, or controvert their views, either for, or against, the identity of the disease as it appears in birds and man.

In the first group all the investigators with the exception of Stevenson (1898) found that the disease in birds was due to organisms entirely different from the diphtheria bacillus. The latter, as the result of experiments on diseased fowls with diphtheria antitoxin, stated that "roup," the popular term for fowl diphtheria, was "caused by a specific germ, which appears to me to be identical with the Klebs-Löffler bacillus, and that roup and canker were the same disease, a disease identical with diphtheria in man."

The opinions of the writers recorded in the second group were divided. Harrison (p. 8) inoculated five healthy fowls with human diphtheria bacilli by scratching the throat, and rubbing in 24 hour cultures. Though these cultures were fully virulent to guinea-pigs the fowls were in no way affected. He was unable to infect guinea-pigs or rabbits with the membranes of bird diphtheria. Finally he reports "we have made cultures from over 200 fowls which died of diphtheria, or were killed in certain stages of the disease, but we have not met with the Klebs-Löffler bacillus in any of them, and consequently we cannot believe in the identity of the human and avian disease."

From his own extensive observations Guérin (1901, 1903) has also arrived at the conclusion that avian and human diphtheria are due to entirely different organisms.

With few exceptions, therefore, all the authors who have made extensive experimental investigations on this subject are of the opinion that human and avian diphtheria are different diseases due to different organisms.

Bacilli morphologically resembling diphtheria bacilli have been found in birds by various investigators. Gordon Sharp (1900) found such organisms, and thought that they were diphtheria bacilli, but of less virulence than those found in man. Gallez (1896) had the same experience, and came to the same conclusions. Turner (1900) isolated from birds with diphtheria a bacillus morphologically resembling the diphtheria bacillus. Guérin (1903) in 78 examinations once found a similar organism, and Malvoz (quoted by Guérin) also found one. Macfadyen and Hewlett (1900) isolated and cultivated bacilli, morphologically resembling diphtheria bacilli, from healthy pigeons and others suffering from pigeon "canker."

They describe these organisms as resembling diphtheria bacilli in size and parallel arrangement. They retain Gram's stain, and show polar bodies by Neisser's method. Growth on serum varies, some examples produce dry and abundant growth like the xerosis bacillus, and others moist colonies like the diphtheria bacillus. These organisms produce indol and acid in broth, and are non-pathogenic to guinea-pigs.

Harrison (1901) also found a diphtheria-like bacillus in the throats of normal pigeons.

I have examined by means of swabs the throats of various birds[1] and found the following diphtheria-like organisms.

10. *Bacillus diphtheroides gallinarum.* (Plate XVI, Fig. 17.)

Origin. From the throat of a fowl. There was a hard tumour on the side of the left mandible, but the bird was otherwise normal.

On *serum* the colonies after 24 hours' growth resemble those of the diphtheria bacillus. Later the margins become crenated. In the first cultures the *organisms* were long, curved, and clubbed, with 3 or 4 well marked polar bodies. Slight swellings were present round the polar bodies. The rest of the protoplasm stained lightly, but slight signs of segmentation were present. They resembled closely the diphtheria bacillus shown on Plate XIV, Fig. 5. In subcultures well marked segments

[1] The birds examined were three fowls (*Gallus domesticus*), two Redpolls (*Acanthes rufescens*), two Pied Wagtails (*Motacella lugubris*), two Partridges (*Perdix cinerea*), two Knots (*Tringa canutus*), one Greenfinch (*Ligurinus chloris*), one Cuckoo (*Cuculus canorus*), one Cockateel (*Calopsittacus novae-hollandiae*), one Crested Mynah, and one Indian Ring-necked Parrakeet. The swabs were kindly procured for me by Dr E. Chichester, of Colchester.

are seen. These organisms are non-motile, and retain the stain deeply by Gram's method. On *agar* small, filmy, transparent, gray colonies are formed. The organisms are very long, thick, curved, clubbed, and well segmented, but no polar bodies are present. On *agar stab cultures* an almost transparent film is formed on the surface, and minute round colonies along the needle track. On *gelatin* after five days' growth minute, round, almost transparent colonies appear. On *gelatin stab cultures* there is very little surface growth, and a very scanty growth of minute colonies along the needle track. On *alkaline potato-agar* in 24—48 hours *very small, smooth, or slightly granular, rounded, almost transparent colonies* are formed. On *acid potato-agar* the growth is similar. No visible growth occurs on *potato*. *Broth* remains clear and there is a slight granular deposit. *Glucose broth* shows a *neutral, or slightly alkaline reaction*. *Indol* is produced. Non-pathogenic to guinea-pigs.

As will be seen from the above description this organism very closely resembles the diphtheria bacillus except in its growth on potato-agar, and in the production of an alkaline reaction in glucose broth.

11. *Bacillus cuculi*. (Plate XVI, Fig. 18.)

Origin. Two cultures were obtained, one from the throat of a cuckoo (*Cuculus canorus*), and the other from that of a parrakeet.

On *serum* after 24 hours' growth the colonies closely resemble those of the diphtheria bacillus. The *organisms* varied in appearance, some were of medium length, curved, clubbed, and stained throughout (like the diphtheria bacillus Plate XIV, Fig. 6), others resembled elongated Hofmann's bacilli with a central light band. Shadowy unstained forms were common. In subcultures the resemblance to the uniformly stained diphtheria bacillus is more evident. They are non-motile, retain Gram's stain, and show small terminal polar bodies by Neisser's method. On *agar large, smooth, white, dome-shaped colonies* are formed in 24 hours. The organisms are long, much curved, clubbed, and well segmented, but show no polar bodies. On *agar stab cultures* an extensive white heaped up surface growth occurs and numerous colonies along the line of puncture. On *alkaline potato-agar round, smooth, dome-shaped, opaque, white colonies* appear. The colonies on *acid potato-agar* after 24 hours' growth are similar, but after 4 days' growth a *thin, filmy, broad expansion* appears round the central mass. On *gelatin* no growth was obtained, and on *potato* no visible growth occurred. *Broth* becomes slightly cloudy, and there is a finely granular deposit. After 48 hours the reaction of *glucose broth* is *neutral*. Non-pathogenic.

This organism differs to some extent in its morphology from the diphtheria bacillus, and differs from it in its growth on agar, and potato-agar and in its reaction in glucose broth.

These observations confirm those of some of the investigators

mentioned, who found in birds organisms morphologically resembling diphtheria bacilli, but differing from them in certain respects.

The *Bacillus xerosis* and the *Bacillus xerosis canis* are the only ones of all the organisms described which die out rapidly in culture. The others remain alive from 30—60 days on serum at room temperature.

No mention has been made in the foregoing account of certain organisms resembling to some extent the diphtheria bacillus in morphological and staining characters, but which differ markedly in having large wrinkled coherent colonies on serum. In such cases mistakes in diagnosis can only be made if instead of the examination of separate colonies, the surface of the medium is scraped, or smear preparations made from many colonies.

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 the 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 (2 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 during any outbreak.

5. Of the 113 examples of the diphtheria bacillus tested for virulence during this outbreak, 87 were fully virulent, and 25 were completely devoid of virulence. One virulent bacillus for reasons explained did not kill the inoculated animal till the 12th day (p. 282). *No partially attenuated bacilli have been found.*

6. In the majority of persons in whom diphtheria bacilli were found, who had recently been in contact with cases of the disease, the bacilli were virulent.

7. Non-virulent bacilli were discovered in 1—2 out of every hundred persons examined, whether contacts or non-contacts. The proportion of persons infected with this organism is therefore the same amongst contacts and persons who have not recently been in contact with the disease.

8. *The absence of polar bodies is no indication of a want of virulence in diphtheria bacilli, and their presence is no indication of the possession of virulence.*

9. Hofmann's pseudo-diphtheria bacillus is a very common inhabitant of the mouths of poorer class children. It is less common amongst adults, even of the same class. *The proportion of persons infected with this organism bears no relation to the proportion infected with the virulent diphtheria bacillus. Notified persons and infected contacts harboured this organism in the same proportions as the healthy school children with whom they had been associated.*

Examples of the Hofmann's bacillus isolated from the first cultures obtained from diphtheria cases were totally non-virulent to guinea-pigs. There is no evidence that it is in any way pathogenic to man.

The distribution of this bacillus points to the conclusion that it is carried from mouth to mouth in the same ways as the diphtheria bacillus, and therefore its widespread prevalence in schools attended by the 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.

10. Organisms morphologically resembling diphtheria bacilli are not infrequently found in the throats of healthy persons, and require careful examination by culture before they can be identified.

11. The xerosis bacillus is a common inhabitant of the normal conjunctival sac, and organisms closely resembling it are present in the eyes of some animals.

12. Virulent diphtheria bacilli have undoubtedly been found in ear discharges, but *diphtheria-like organisms appear to be extremely common in the ear discharges of scarlet fever patients, and in the ears of normal persons. Consequently no conclusions as to the frequency of the diphtheria bacillus in the ears of scarlet fever patients can be made without the thorough examination of any organisms which may be discovered both by cultural, and virulence, tests.*

13. Diphtheria-like organisms occur in the throats of healthy birds.

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ADDENDUM.

In the middle of April another employee in the Post Office was found to be harbouring diphtheria bacilli. Like those previously discovered amongst these persons the bacilli were non-virulent.

(a) = Notified case. Diphtheria bacilli found.
(b) = "Contact" infected with diphtheria bacilli.
Δ = Virulent diphtheria bacillus.
Λ = Non-virulent diphtheria bacillus.
* = Hofmann's pseudo-diphtheria bacillus found.
SS = Small swelling in subcutaneous tissue of guinea-pig.
MS = Moderate ,, ,, ,, ,,
LS = Large ,, ,, ,, ,,
\+ = Death of guinea-pig.

N = No result. Guinea-pig not affected.
dark red = Suprarenals very dark in colour.
red = Suprarenals deep red in colour.
pink = Suprarenals pink in colour.
present = Pseudo-diphtheria bacilli found on one or more examinations.

In I, II, III or IV days, *i.e.* within 24, 48, 72 or 96 hours.

No.	Initials	Results of consecutive examinations	Days during which diphtheria bacilli remained in throat	Reaction in glucose broth	Animal inoculations						Results of Autopsy			Pseudo-diphtheria bacillus
					Wt. of guinea-pig in grammes	Dose of 48 hours broth culture in c.c.	Results. Days I	II	III	IV	Oedema	Suprarenals	Fluid in pleura	
St Matthew's School (Girls)														
1	A.S. (a)	O.O.Δ.Δ.Δ.Δ.O * * *	72	acid	170	·2	MS	+			extensive haemorrhagic	dark red	trace	present
2	W.B. (a)	Δ.O.O.O	8	,,	170	·2	SS	+			extensive	red	-	-
3	G.G. (a)	Fatal. No examinations												
4	K.M. (b)	Λ.O.O.O * * *	8	acid	180	·2	N	N	N	14th day N	-	-	-	present
					200	2·0	N	N	N	N	-	-	-	-
5	G.C. (b)	Δ.Δ.Δ.Δ.O.Δ.O.Δ.Δ.Δ.Δ.O.O. O.Δ.O.O.O	44	,,	240	·2	SS	+			slight	dark red	-	-
6	W.L. (b)	Δ.O.O.O.O * * * *	8	,,	210	·1	LS	+			extensive	dark red	·8 c.c.	present
7	E.P. (b)	Δ.O.O.O * * * *	8	,,	235	·1	MS	+			,,	,,	3·2 c.c.	present
8	C.S. (b)	Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.O.O.O * * * *	29	,,	180	·2	MS	LS	+		very extensive	,,	-	present
9	R.S. (b)	Δ.Δ.Δ.O.Δ.O.O.Δ.O.O	83	,,	280	·1	MS	+			extensive haemorrhagic	,,	trace	-
St Matthew's School (Infants)														
10	H.O. (a)	Δ.O.O.O * * * *	21	,,	not tested						-	-	-	present
11	F.A. (a)	Δ.O.O.O * * *	17	,,	230	·1	MS	+			extensive	dark red	4·6 c.c.	present
12	F.E. (a)	Δ.O.Δ.O.Δ.O.O.O.O.O * *	63	,,	160	·2	SS	+			extensive haemorrhagic	,,	-	present
13	F.H. (a)	Δ. Fatal	-	,,	170	·2	MS	LS	+		extensive	,,	-	-

14	M.F. (a)	Δ.O.O.O * * *	28	acid	190	·2	MS	+			extensive	dark red	·9 c.c.	present
15	N.W. (a)	Δ.Δ.Δ.Δ.O.O.O * * * *	24	,,	430	·3	SS	+			extensive haemorrhagic	,,	-	present
16	D.H. (b)	Δ.Δ.Δ.O.O.Δ.Δ.O.O.O.O * * * * *	29	,,	225	·1	MS	LS	LS	+	,,	red	3·4 c.c.	present
17	D.E. (b)	Δ.Δ.O.O.O.O * * *	14	,,	225	·1	SS	+			,,	red	trace	present
18	V.L. (b)	Δ.O.O.Δ.Δ.Δ.Δ.O.Δ.Δ.Δ.O.O.O * * * * *	41	,,	270	·1	MS	LS	+		,,	dark red	·4 c.c.	present
19	M.P. (b)	Δ.Δ.Δ.Δ.O.O.O * * *	20	,,	240	·1	SS	+			extensive	,,	1·0 c.c.	present
20	T.E. (b)	Δ.Δ.Δ.Δ.O.O.O	22	,,	200	·1	SS	SS	+		slight	red	-	-
21	H.W. (b)	Λ.Λ.Λ.Λ.Λ.Λ.Λ.O.Λ.O.O.Λ.Λ. * Λ.O.Λ.O.O.O * * * * * *	52	,,	200 †200	·2 2·0	N SS	N N	N N	14th day N N	- -	- -	- -	present -
22	B.T. (b)	Δ.O.O.O * * *	9	,,	235	·2	LS	+			extensive haemorrhagic	dark red	2·3 c.c.	present
23	S.C. (b)	Δ.Δ.Δ.Δ.Δ.Δ.Δ.O.Δ.O.Δ.Δ.O. * * * * * * * O.Δ.O.Δ.O.O.O * * * *	51	,,	300	·1	MS	LS	LS	+	extensive	,,	-	present
24	G.T. (a)	Δ.O.O.O * * * *	17	,,	150	·2	MS	+			extensive haemorrhagic	red	-	present
25	S.M. (b)	Δ.O.O.O * * *	9	,,	150	·1	SS	+			extensive	dark red	-	present
26	F.S. (b)	Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.O.Λ.O. Λ.Λ.Λ.Λ.Λ.Λ.Λ.O.Λ.Λ.O.Λ.Λ. Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ. Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.O.Λ	122	,,	180 ‡210	·2 2·0	N N	N N	N N	14th day N N	- -	- -	- -	- -
27	W.O. (b)	Λ.O.O.O * * *	9	,,	200	·2	N	N	N	14th day N	-	-	-	present
28	A.M. (b)	Λ	-	,,	340	2·0	N	N	N	14th day N	-	-	-	-
29	L.R. (a)	O.Δ.Δ.O.O.O * * * *	30	,,	170	·2	MS	+			slight	dark red	-	present
30	M.H. (a)	Δ. Fatal	-	,,	410	·2	SS	MS	+		extensive haemorrhagic	,,	2·5 c.c.	-
31	J.M. (a)	Δ.Δ.Δ.Δ.Δ.Δ.Δ.O.O.Δ.O.Δ.O. O.O.O.O.O.Δ.O.O	58	,,	210	·2	SS	+			moderate	,,	-	-
32	A.M. (b)	Δ.Δ.O.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ. * * * * * * * * * * Δ.O.Δ.Δ.O.Δ * *	47	,,	250	·2	SS	+			extensive	,,	12·0 c.c.	present
33	W.R. (a)	Δ.O.Δ.O.O.O.O * * * * * *	32	,,	225	·2	LS	+			,,	,,	1·0 c.c.	present

† Bacilli from 7th examination.

‡ Bacilli from 28th examination.

No.	Initials	Results of consecutive examinations	Days during which diphtheria bacilli remained in throat	Reaction in glucose broth	Animal inoculations						Results of Autopsy			Pseudo-diphtheria bacillus
					Wt. of guinea-pig in grammes	Dose of 48 hours broth culture in c.c.	Results. Days I	II	III	IV	Oedema	Supra-renals	Fluid in pleura	
34	C.L. (a)	Δ. Fatal	-	acid	195	·2	MS	+			extensive	dark red	3·2 c.c.	-
35	F.B. (a)	Δ.O.Δ.Δ.Δ.Δ.O.O.O.O.O	27	,,	210	·2	SS	MS	+		,,	,,	-	-
36	A.C. (a)	Δ. Fatal	-	,,	220	·2	MS	+			,,	red	6·3 c.c.	-
37	R.G. (a)	Δ.Δ.Δ.O.O.O.O.O	25	,,	150	·2	SS	+			,,	dark red	2·0 c.c.	-
38	J.G. (a)	Δ.O.O.O.O.O	6	,,	190	·2	LS	+			,,	,,	4·0 c.c.	-
Sturton Street School														
39	A.L. (a)	Δ.O.O.O * * * *	14	,,	260	·1	SS	MS	+		,,	,,	1·3 c.c.	present
40	G.S. (b)	Δ.Δ.Δ.O.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ. * * * * Δ.Δ.Δ.Δ.O.O.Δ.Δ.O.Δ.O.O.O * * * * * * * * *	27	,,	260	·3	LS	LS	+		extensive haemorrhagic	,,	6·3 c.c.	present
41	J.T. (a)	Δ.Δ.Δ.O.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ. * * Δ.O.Δ.Δ.Δ.O.O.O * *	58	,,	210	·2	MS	+			extensive	,,	5·5 c.c.	present
42	B.T. (b)	Λ.Λ.Λ.O.O.Λ.Λ.Λ.Λ.Λ.Λ.Λ. * * * * Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.O.Λ. * * * * O.O.O * * *	62	,,	190 †310	·2 2·0	N N	N N	N N	14th day N N	- -	- -	- -	present -
43	M.E. (a)	Δ.O.Δ.O.O.Δ.O.Δ.O.Δ.Δ.O.O. O.O.O.O	32	,,	270	·2	LS	LS	+		extensive haemorrhagic	dark red	4·5 c.c.	-
44	S.E. (a)	Δ.Δ.O.O.O.O * *	22	,,	200	·2	MS	+			extensive	,,	·5 c.c.	present
Hospital														
45	F.H. (a)	Δ.Δ.O.O	18	,,	200	·2	SS	+			moderate	,,	-	-
46	C.B. (a)	Δ.O.O.O.O	15	,,	200	·2	MS	+			extensive	,,	-	-
47	M.S. (a)	Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.O.O.O	56	,,	220	·2	SS	LS	+		,,	,,	3·5 c.c.	-
48	R.C. (a)	Δ.O.Δ.Δ.Δ.Δ.O.O.Δ.O.O * * *	41	,,	275	·2	MS	+			extensive haemorrhagic	red	4·8 c.c.	present

48	A.R. (a)	Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.Λ.O.O.O	51	acid	250	·2	N	N	N	N (14th day)	-	-	-	-
50	H.R. (a)	Δ.Δ.Δ.O.O.Δ.O.O.O.Δ.O.Δ.Δ. Δ.Δ.Δ.O.O	99	,,	250	·2	SS	MS	+		extensive	dark red	4·5 c.c.	present
51	G.R. (b)	Δ.Δ.Δ.Δ.O.Δ.Δ.O.O.O.O	48	,,	250	·2	LS	LS	+		,,	,,	-	-
Sanatorium														
52	A.W. (a)	Δ.Δ	-	,,	250	·2	MS	+			extensive	,,	-	-
53	T.S. (b)	Δ.Δ.Δ.Δ.O	44	,,	200	·2	SS	MS	+		,,	,,	-	-
54	R.F. (a)	Δ.O.O.O	33	,,	210	·2	MS	+			,,	,,	9·0 c.c.	present
55	D.W. (b)	Δ. Fatal	-	,,	250	·2	MS	LS	+		,,	,,	5·6 c.c.	-
56	E.H. (b)	Δ.O.O.O	12	,,	320	·2	MS	LS	+		,,	,,	3·4 c.c.	-
57	B.M. (b)	Δ.O.O.O	13	,,	320	·2	LS	+			extensive haemorrhagic	,,	-	-
58	H.H. (b)	Δ.Δ.O.O.O.O	12	,,	350	·2	LS	LS	+		extensive	,,	-	-
59	E.H. (b)	Δ.O.Δ.O.O.O	10	,,	330	·2	LS	LS	+		,,	,,	-	-
60	L.B. (a)	Δ.Δ.O.O.O	25	,,	320	·2	SS	+			slight	red	7·5 c.c.	-
St Matthew's School (Infants—Autumn)														
61	F.B. (a)	Δ. Fatal	-	,,	300	·3	SS	LS	LS	+	extensive	red	2·5 c.c.	-
62	K.R. (a)	Δ.O.O.Δ.O.O.O.O.O	30	,,	420	·3	SS	LS	+		,,	dark red	-	-
63	H.H. (a)	Δ.O.O.Δ.Δ.Δ.Δ.O.O.O.O	31	,,	430	·3	MS	LS	+		,,	red	9·7 c.c.	-
64	B.P. (a)	Δ.Δ.Δ.Δ.O.O.O	27	,,	250	·3	MS	+			,,	dark red	1·0 c.c.	-
65	L.K. (b)	Δ.Δ.Δ.O.O	28	,,	450	·3	MS	LS	+		moderate	pink	6·2 c.c.	present
66	H.L. (b)	Δ.Δ.O.Δ.O.O.O	28	,,	450	·3	SS	+			extensive	,,	1·2 c.c.	present
67	B.T. (b)	Δ.Δ.O.O.Δ.O.O.O	33	,,	400	·3	LS	+			,,	dark red	-	-
68	N.M. (b)	Δ.Δ.O.O	57	,,	350	·3	SS	+			,,	,,	2·2 c.c.	present
69	H.M. (b)	Δ.O.Δ	?	,,	246	·3	MS	+			,,	,,	-	-
70	G.P. (a)	Δ.Δ.Δ.Δ.O.O.O.O.O	18	,,	430	·3	MS	LS	+		,,	,,	-	-

† Bacilli from 19th examination.

No.	Initials	Results of consecutive examinations	Days during which diphtheria bacilli remained in throat	Reaction in glucose broth	Animal inoculations						Results of Autopsy			Pseudo-diphtheria bacillus
					Wt. of guinea-pig in grammes	Dose of 48 hours broth culture in c.c.	Results. Days I	II	III	IV	Oedema	Supra-renals	Fluid in pleura	
Catherine Street School														
71	L.S. (a)	Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.Δ.0.Δ.0.0.0.0	65	acid	250	·2	MS	+			extensive	red	-	-
72	D.G. (a)	Δ.Δ.0.Δ.Δ.Δ.Δ.Δ.Δ.0.0.Δ.0.0.0 * * * * * *	33	,,	200	·3	SS	MS	+		,,	dark red	-	present
73	A.M. (a)	Δ.Δ.Δ.Δ.0.0.Δ.Δ.0.0.0	30	,,	250	·3	SS	+			moderate	,,	3·2 c.c.	-
74	E.B. (b)	Λ.0.0.0	13	,,	190	·3	N	N	N	14th day N	-	-	-	-
75	E.K. (b)	Δ.Δ.0.0.0 * * *	21	,,	245	·3	SS	+			extensive	dark red	3·2 c.c.	present
76	A.W. (b)	Δ.Δ.Δ.Δ.Δ.0.0.0.0.0.0	23	,,	200	·3	SS	+			moderate	,,	-	-
77	E.G. (b)	Δ.0.0	13	,,	160	·3	SS	+			extensive	,,	-	-
78	M.H. (b)	Δ.0.Δ.Δ.0.0.0.0 * *	23	,,	225	·3	SS	MS	+		extensive haemorrhagic	red	-	present
79	A.S. (b)	Δ.Δ.Δ.0.Δ.0.0.0 * *	23	,,	205	·3	SS	LS	+		extensive	red	-	present
80	M.S. (b)	Δ.Δ.0.Δ.Δ.0.0.Δ.0.0.0 * *	40	,,	160	·3	MS	+			moderate	dark red	-	present
81	N.S. (b)	Δ.Δ.0.Δ.0.0.0.0 * * *	19	,,	200	·3	SS	+			extensive	,,	1·2 c.c.	present
82	N.B. (b)	Λ.0.0.0	13	,,	205	2·0	N	N	N	14th day N	-	-	-	-
83	M.A. (b)	Λ.Λ.Λ.0.0.0 *	16	,,	200	·3	N	N	N	14th day N	-	-	-	present
84	W.C. (b)	Δ.0.0	47	,,	250	·3	SS	+			extensive	dark red	-	-
85	G.T. (b)	Δ.0.0.0	8	,,	200	·3	SS	SS	SS	12th day +	†slight	pink	5·8 c.c.	-
86	F.E. (b)	Δ.0.0.0	16	,,	230	·3	MS	+			extensive haemorrhagic	,,	-	-
87	L.G. (a)	0.Δ.Δ.Δ.0.0.0 * * *	19	,,	200	·3	SS	+			,,	dark red	-	present
88	K.T. (a)	Δ.0.Δ.0.0.0 * * * * * *	18	,,	150	·3	SS	MS	+		moderate	,,	-	present
89	G.T. (b)	Λ.0.0.0	20	,,	220	·3	SS	N	N	N	-	-	-	-
Abbey School														
90	M.A. (b)	Λ	?	,,	245	2·0	N	N	N	N	-	-	-	-
New Street School														
91	C.H. (b)	Λ.Λ.0.0.0	10	,,	200	2·0	N	N	N	N	-	-	-	-

92	E.O. (a)	Δ.Δ.Δ.Δ	81	acid	200	·2	MS	+			moderate	dark red	trace	-
93	D.O. (a)	Δ.Δ.O.O * * * *	57	,,	260	·2	SS	+			,,	,,	-	present
94	G.O. (b)	Δ.O.O * * *	?	not tested										present
Post Office														
95	A.O. (a)	Δ.Δ.Δ.Δ.Δ.Δ.O.O.Δ.O.O.O	16	acid	170	·3	MS	+			moderate	dark red	1·3 c.c.	-
96	J.H. (a)	Λ.Λ.O.Λ.Λ.Λ.O.Λ.O.O.Λ.O.O.O * * * * *	15	,,	300	·2	N	N	N	14th day N	-	-	-	present
					290	2·0	N	N	N	N	-	-	-	-
97	F.H. (b)	Λ.Λ.Λ.Λ.O.O.O.O * * * *	8	,,	260	2·0	SS	N	N	N	-	-	-	present
98	Y.B. (b)	Λ.Λ.Λ.Λ.O.O.O	9	,,	300	2·0	N	N	N	N	-	-	-	-
99	R.L. (b)	Λ.O.Λ.O.O.O * * * *	8	,,	200	2·0	N	N	N	N	-	-	-	present
Immediate contact unknown														
100	W.O. (a)	Δ.O.O.O * *	12	,,	210	·2	LS	+			extensive haemorrhagic	dark red	-	present
101	F.P. (a)	Δ. Fatal	-	,,	220	·2	MS	MS	LS	+	extensive	red	6·3 c.c.	-
102	M.S. (a)	Δ.Δ.O.O.Δ.Δ.Δ.O.O.O	35	,,	200	·2	LS	+			,,	dark red	1·5 c.c.	-
103	D.C. (a)	Δ. Fatal	-	,,	205	·2	MS	LS	+		,,	,,	-	-
104	O.O. (b)	Δ.Δ.Δ.Δ.Δ.O.O	70	,,	198	·3	MS	+			,,	,,	-	-
105	M.A. (a)	Δ.Δ.O.O.Δ.O.Δ.O.O.O.O	58	,,	245	·3	SS	+			haemorrhagic	,,	1·2 c.c.	-
106	G.W. (a)	Δ. Fatal	-	,,	245	·3	SS	+			extensive	,,	-	-
107	A.R. (a)	Λ.Λ.Λ.Λ.O.O.O * * * *	11	,,	210	·2	N	N	N	14th day N	-	-	-	present
					200	2·0	N	N	N	N	-	-	-	-
108	M.R. (b)	Λ.Λ.O	20	,,	200	2·0	N	N	N	N	-	-	-	-
109	M.R. (b)	Λ	-	,,	not tested									
110	W.R. (b)	Λ.Λ.O * *	20	,,	205	2·0	N	N	N	N	-	-	-	present
111	D.R. (a)	O.Λ.Λ.Λ.Λ.O.O.Λ.O.O.O * * * * * * * * *	15	,,	220	2·0	N	N	N	N	-	-	-	present
112	E.Y. (a)	O.O.Λ.O.Λ	?	,,	210	2·0	N	N	N	N	-	-	-	-
113	A.I. (a)	Λ.Λ.O.O	8	,,	230	2·0	N	N	N	N	-	-	-	
114	N.E. (a)	Λ.Λ.Λ.O	11	,,	300	2·0	N	N	N	N	-	-	-	-
115	J.C. (a)	Λ.O.O.O	1	,,	200	2·0	N	N	N	N	-	-	-	-

† See note in text (p. 282).

PUBLICATIONS RECEIVED.

BIFFI, U. and CARTY, C. L. (1903), *Saneamiento de Lima. Proyectos.* Lima: Librería é imprenta Gil. 21 × 15 cm.[1] Paper. 108 pp. With maps and plans of buildings.

FOWLER, G. J. (1904), *Disposal of sewage and other refuse. The application of chemical analysis to the study of the biological processes of sewage purification.* Lecture delivered at the Public Health Laboratory, University of Manchester. Manchester: Sherratt and Hughes. 21 pp. (Price unbound 1*s.* 6*d.*) 24 × 18 cm.

GOULD, G. M. (1904), *Biographic Clinics*, vol. II. *The origin of the ill health of George Eliot, George Henry Lewes, Wagner, Parkman, Jane Welch Carlyle, Spencer, Whittier, Margaret Fuller Ossoli, and Nietzsche.* Philadelphia: P. Blakiston's Son and Co. 392 pp. Cloth. 20 × 13 cm.

GRASSBERGER, R. and SCHATTENFROH, A. (1904), *Ueber die Beziehungen von Toxin und Antitoxin.* Leipzig und Wien: Franz Deuticke. 103 pp. (Price unbound 3 Marks.) 26 × 18 cm. (Important experimental and critical contributions to the subject of the relations between toxin and antitoxin.)

LAVERAN, A. (1903), *Prophylaxie du paludisme.* (Volume belonging to a series entitled "Encyclopédie scientifique des aide-mémoire." Paris: Masson et Cie, and Gauthier-Villars. 209 pp. With 20 figures in the text. Paper. 16 × 12 cm.

MCDILL, J. R. and WHERRY, W. B. (1904), *A report on two cases of a peculiar form of hand infection, due to an organism resembling the Koch-Weeks bacillus.* Department of Interior. Bureau of Government Laboratories. Biological Laboratory. 1903. No. 10. Manila: Bureau of Public Printing. 20 pp. 2 plates. 23 × 15 cm.

MERRILL, E. D. (1904), *New or noteworthy Philippine Plants.—The American element in the Philippine Flora.* Department of Interior. Bureau of Government Laboratories. Manila: Bureau of Public Printing. 1903. No. 6. 36 pp. 23 × 15 cm.

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New York State Hospital for the care of crippled and deformed children. Third annual report, for the year ending September 3, 1903. Albany: J. B. Lyon Co. 28 pp. 7 plates. 23 × 15 cm.

NIVEN, J. (1904), *Food and drink in relation to disease. Feeding in relation to the health of the young.* Lecture delivered at the Public Health Laboratory, University of Manchester. Manchester: Sherratt and Hughes. 42 pp. (Price unbound 1*s.* 6*d.*) 24 × 18 cm.

PRESCOTT, S. C. and WINSLOW, C. E. A. (1904), *Elements of Water Bacteriology with special reference to sanitary water analysis.* New York: John Wiley and Sons. London: Chapman and Hall, Ltd. 162 pp., including 14 pp. of bibliography. (Price $1.25.) Cloth. 16 × 12 cm.

SHERMAN, P. L. (1903), *The gutta percha and rubber of the Philippine Islands.* Department of Interior. Bureau of Government Laboratories. Chemical Laboratory. 1903. No. 7. Manila: Bureau of Public Printing. 43 pp. 41 figures and maps. 23 × 15 cm.

WOOLLEY, P. G. (1904), *Report on some pulmonary lesions produced by the Bacillus of hemorrhagic septicaemia of carabaos.* Department of Interior. Bureau of Government Laboratories. Biological Laboratory. 1903. No. 12. Manila: Bureau of Public Printing. 11 pp. 23 × 15 cm.

WOOLLEY, P. G. and JOBLING, J. W. (1904), *A report on hemorrhagic septicaemia in animals in the Philippine Islands.* Department of Interior. Bureau of Government Laboratories. Biological Laboratory. 1903. No. 9. Manila: Bureau of Public Printing. 21 pp. 2 charts. 23 × 15 cm.

[1] The size of the publications is given roundly in centimetres.—*Ed.*

DIPHTHERIA BACILLI FROM SERUM CULTURES.

Fig. 1.

Irregularly beaded form.
Subculture 24 hours.

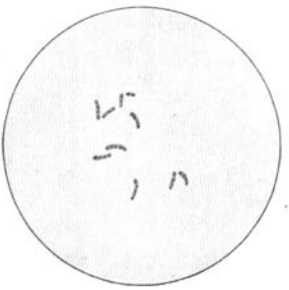

Fig. 2.

"Streptococcal" form.
First culture 24 hours.

Fig. 3.

Segmented form.
First culture 24 hours.

Fig. 4.

Segmented form.
First culture 24 hours.

Fig. 5.

Thin segmented form.
First culture 24 hours.

Fig. 6.

Uniformly stained form.
Subculture 24 hours.

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

https://doi.org/10.1017/S0022172400002163 Published online by Cambridge University Press

DIPHTHERIA-LIKE BACILLI FROM SERUM CULTURES.

Fig. 7.

Hofmann's bacillus (Pseudo-diphtheria type). First culture 24 hours.

Fig. 8.

Typical Hofmann's bacillus. Subcultures from Pseudo-diphtheria type 24 hours.

Fig. 9.

Bacillus diphtheroides citreus. First culture 24 hours.

Fig. 10.

Bacillus diphtheroides brevis. First culture 24 hours.

Fig. 11.

Bacillus maculatus. First culture 24 hours.

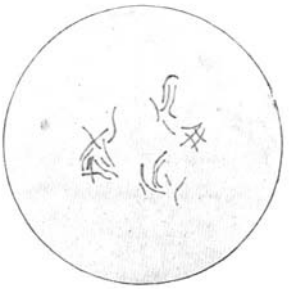

Fig. 12.

Bacillus diphtheroides liquefaciens. First culture 24 hours.

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DIPHTHERIA-LIKE BACILLI FROM SERUM CULTURES.

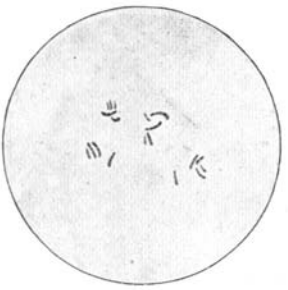

Fig. 13.

Bacillus xerosis.
First culture 30 hours.

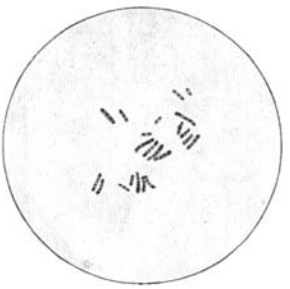

Fig. 14.

Bacillus xerosis canis.
First culture 48 hours.

Fig. 15.

Bacillus auris.
First culture 48 hours.

Fig. 16.

Bacillus ceruminis.
First culture 30 hours.

Fig. 17.

Bacillus diphtheroides gallinarum.
First culture 24 hours.

Fig. 18.

Bacillus cuculi.
First culture 12 hours.

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COLONIES ON ALKALINE POTATO AGAR.

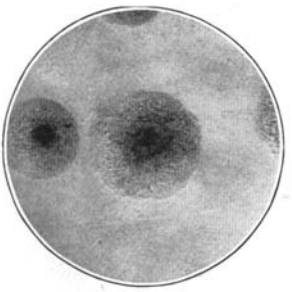

Fig. 1.

B. diphtheriae. Type (a). 48 hours.

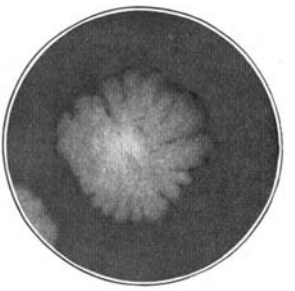

Fig. 2.

B. diphtheriae. Type (a), rare form. 48 hours.

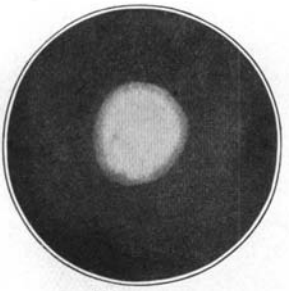

Fig. 3.

B. diphtheriae. Type (b). 48 hours.

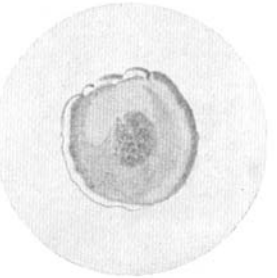

Fig. 4.

B. diphtheriae. Type (b). 48 hours. Drawn under Zeiss' binocular dissecting microscope. A.o. lens. No. 4 oc.

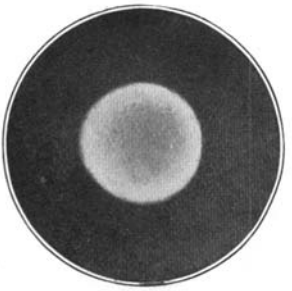

Fig. 5.

B. ceruminis. 48 hours. The majority of diphtheria-like organisms including Hofmann's bacillus produce colonies of this type.

Fig. 6.

B. maculatus. 48 hours.

These colonies were (except Fig. 4) all photographed by Mr Walter Mitchell of this Laboratory: Fig. 1 by direct and the others by oblique illumination, all of the same magnification.

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