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DIPHTHERIA AND PSEUDO-DIPHTHERIA.

A Report to the New York City Health Department on the Bacteriological Examination of 5611 Cases of Suspected Diphtheria, with the results of other Investigations upon the Diphtheria and Pseudo-Diphtheria Bacillus. From the Bacteriological Laboratory.

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Statistics of Bacteriological Examinations in Cases of Suspected Diphtheria.

FROM May 4th, 1893, to May 4th, 1894, there were 5611 cases of suspected diphtheria subjected to bacteriological examination. In 3255 of these the Loeffler diphtheria bacilli were found to be present, and these cases were thus proven to be true diphtheria. In 1540 no diphtheria bacilli were present in the cultures, and as these had been carefully made at an early period of the disease, the cases from which they were taken may be considered as proven not to have been true diphtheria. In 816 cases, although no diphtheria bacilli were found in the cultures, yet, either because they were made after the fourth day of the disease, or the exudate was imperfectly obtained from the throat, or the culture media had become contaminated, or was too dry, the cases from which the cultures were obtained, although probably not diphtheria, were considered to be of a doubtful nature, as far as the bacteriological examination was concerned.

Thus we find in 5611 cases of suspected diphtheria that about 58

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per cent. were proven to be true, 27 per cent. to be false, or pseudo-diphtheria, and 15 per cent. to be of a somewhat doubtful character. It would probably be just to consider that 60 per cent. were true, and 40 per cent. were false diphtheria.

Sex and Age and Mortality in the Cases of True Diphtheria.

In a large percentage of the cases, in which the sex was determined, there were 54 per cent. females and 46 per cent. males, a fairly even division. The statistics reveal some interesting facts as to the influence of age upon the occurrence of true diphtheria and its mortality. The ages of persons attacked ranged between three weeks and seventy years. The number of cases increased with each twelve months of life up to the fourth, and then gradually diminished. The mortality was highest in the first two years of life, and then steadily diminished until adult life was reached, when it again slowly increased. The ages and mortality were determined in 1625 cases, and were as follows :—

Age.	No. Cases.	Mortality.	Age.	No. Cases.	No. Cases per year average.	Mortality.
1st 12 months...	24	45%	7th to 10th year	292	97 +	15%
2nd ,, ...	109		10th to 15th year	117	23 +	5%
3rd ,, ...	233		15th to 20th year	20	4	—
4th ,, ...	258		20th to 30th year	41	4 +	20%
5th ,, ...	192	33%	30th to 50th year	13	13/20	—
6th ,, ...	163					
7th ,, ...	163					

Age and Mortality in False or Pseudo-Diphtheria.

It has been the general rule of the Department to take no further cognizance of cases of false diphtheria after the culture has demonstrated the absence of the diphtheria bacilli.

In order, however, to compare the mortality and contagiousness of cases of false diphtheria with the true, 450 false cases were carefully investigated by sanitary inspectors detailed for this work. These cases comprised 300 occurring in the fall months, and 150 occurring in March and April. The cases were taken in consecutive order, and are believed to be average cases.

The ages were determined in 628 cases, and the mortality in 450 cases.

Age.	No. Cases.	Mortality all Cases.	Uncomplicated with scarlet fever.	Age.	No. Cases.	No. Cases per year average.	Mortality.
1st 12 months	10	6 %	5¼ %	7th to 10th year	108	36	0
2nd ,,	21			10th to 15th year	90	16	0
3rd ,,	57			15th to 20th year	45	9	0
4th ,,	74			20th to 30th year	75	7½	
5th ,,	43	2 %	0	30th to 50th year	30	1½	2 %
6th ,,	40			Over 50	2	—	
7th ,,	45						

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In the 450 cases investigated there were 42 in which scarlet fever existed as a complication, and of these four died. In the 408 uncomplicated cases there were seven deaths, or 1·7% per cent, as contrasted with 27 per cent. in true diphtheria.

Only two of these seven deaths occurred in persons over five years of age. One of these was a man of seventy years, who was suffering from a marked valvular lesion of the heart, and the other was in a young adult female who died of septicæmia.

The history in brief of the second case was as follows: Three weeks before death the disease began with a swelling of one tonsil and its surrounding tissues; a week later the tonsil was incised, but no pus obtained; about the incision a dirty brown pseudo-membrane formed. Later the tonsil and surrounding tissue became necrotic, and sloughed off, then the ulceration extended to the pharynx and the other tonsil, and was still progressing when the patient died of sepsis and exhaustion.

The five deaths in children under five years of age all occurred in cases in which the larynx was affected, and in three more or less bronchopneumonia developed as a complication.

True and Pseudo-Diphtheria of the Larynx (Membranous Croup).

The statistics as to the location of the disease in the true and false cases is of especial interest. There were 286 of the cases examined in which the disease was entirely or chiefly confined to the larynx or bronchi; of these 283 were in children, and three in adults. In the cultures of 229 of these 286 the characteristic Loeffler diphtheria bacilli were found, and the cases were thus proven to be true diphtheria. Of the 229 cases in which the Loeffler bacilli was found 167 showed no pseudo-membrane or exudate above the larynx, while in the remaining 62, although the larynx was mainly involved, there was also some membrane or exudate present on the tonsils or pharynx. In 57 out of 286 examined no diphtheria bacilli were found, but in 17 of these the cultures were unsatisfactory. Excluding the 17 cases, there were 40 in which the diphtheria bacilli were absent. The disease was confined to the larynx or bronchi in 27 of the 40, while more or less exudate or membrane was present on the tonsils or in the pharynx in 13.

We find, therefore, that of the cases of acute laryngitis in children which have been subjected to bacteriological examination in the laboratory of the Health Department during the past ten months, about 80 per cent. have proven to be undoubtedly cases of diphtheria, and of the remaining 20 per cent. only 14 per cent. were certainly not diphtheritic.

Not only have the bacteriological examinations shown that a large proportion of the cases of acute croupous laryngitis in children (commonly designated by the name membranous croup) are diphtheria, but the Department inspectors have frequently found that these cases were the origin of characteristic pharyngeal diphtheria.

Many experienced physicians still find difficulty in believing that cases in which the exudate or pseudo-membrane is entirely absent from the pharynx and tonsils are true diphtheria, and it is also difficult to persuade parents that such cases are diphtheria. For instance, a child,

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aged five, subject to attacks of bronchitis and slight laryngitis, developed a croupy cough. For diagnostic purposes a culture was made, and the diphtheria bacilli found to be present. It was with the greatest difficulty that the parents could be made to consider the case a serious one, and to separate the children. Under suitable treatment, on the fifth day, the child seemed nearly recovered, and now the parents became sure it was not a case of diphtheria, stopped all precautions, allowed the child to go out, &c. A relapse followed, the laryngeal symptoms increased, and the child died in thirty-six hours of asphyxia, intubation being refused.

The comparatively small number of laryngeal cases examined is partially due to the fact that membranous croup has not been considered a contagious disease, and reports of such cases have not been required by the deputant, and partially due to the custom of the Department inspectors not to make cultures in cases which have been intubated, or which seem so sick that the family might think injury had been done by inserting the swab in the throat. The cases in which no cultures are made are treated as cases of true diphtheria.

The Clinical as contrasted with the Bacteriological Diagnosis.

Upon the blanks accompanying such "culture outfit" a space is left for a record of the clinical diagnosis made by the physician. Upon the blanks received from 1220 cases of true diphtheria, the physician's diagnosis was recorded. In 1011 of the 1220 the case was considered to be diphtheria, in 102 to be doubtful, and in 97 to be probably false. Upon the blanks received from 675 cases in which the diphtheria bacilli were absent, a diagnosis of true diphtheria was made in 50, the rest being all considered as doubtful.

The cases which present all the characteristic appearances of well-developed diphtheria regularly contain in the pseudo-membranes abundant diphtheria bacilli. For instance, in a case like the following a culture is not necessary to determine the diagnosis. A lad aged twelve was seized, two days before inspection, with a slight chill, and felt a soreness in the region of the tonsils, and began to have pain in swallowing. All symptoms had steadily increased. When seen, forty-eight hours after onset of disease, the boy seemed rather apathetic, and was constantly spitting. He said he did not feel much sick, but could not swallow, and found it difficult to breathe freely when lying down. The lymph nodes on right side of neck were swollen, as were also the surrounding tissues, so that the depression beneath the jaw was obliterated.

The tonsils and the margins of the tonsillar pillars were covered by a thin dirty grey adherent pseudo-membrane. The right side of the uvula and a portion of the soft palate was covered by a thin friable pseudo-membrane, which could be removed easily without leaving a bleeding surface.

The faucial pillars and the uvula were so swollen and œdematous that the posterior wall of the pharynx could not be seen. The nostrils and the larynx were free.

It is in the slightly developed cases that a culture is needed. In those

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without cultures it is necessary to be governed more by the history of exposure than the appearance of the throat. In a family where an undoubted case of diphtheria existed any form of sore throat, with or without exudate, occurring either in a child or in an adult, would probably be a case of diphtheria, while a tonsillitis or pharyngitis, perhaps similar in appearance, occurring in a person who has not been exposed to diphtheria, would be in all probability a case of ordinary non-diphtheritic throat inflammation.

The Relation between the Size of the Bacillus and its Virulence.

Some investigators have believed that the degree of virulence possessed by the diphtheria bacilli could, to a certain extent, be judged by their length. The longest bacilli were supposed to be the most virulent, the medium less so, and the shortest little, if at all, virulent. By observing this characteristic, it was thought cultures might become helpful in prognosis. Very careful notes have been made on this point in the examinations of the bacteria from the original serum tubes in 1613 cases. The Loeffler bacilli in 1398 of these were of about the average size, and characteristically stained. In these there was a mortality of about twenty-six per cent. The bacilli in 82 cases were considerably longer than the average. In these there was a mortality of twenty-seven per cent. In 67 cases the Loeffler bacilli were distinctly shorter than the average, and in these there was a mortality of thirty-five per cent. In 66 cases the bacilli were short, not characteristic in shape, and evenly stained; many of them belonged to the class of pseudo-diphtheria bacilli. In these there was a mortality of only twelve per cent.

The results obtained from the examination of 1613 cultures in which this factor was carefully noted, therefore, indicate that in New York the great majority of cases of diphtheria yield in cultures bacilli of medium size, which are characteristic in shape and in manner of staining. In a moderate number the bacilli found are much longer, and in about an equal number they are much shorter. Both the clinical histories and the animal experiments showed that as long as in shape and staining the bacilli are characteristic, nothing as regards their virulence, either in men or animals, can be judged from their length. Those bacilli, on the other hand, which are short, and stain uniformly with methyl blue, usually prove to be of the pseudo-diphtheria type, and have no virulence in animals.

So many inquiries have been sent to the Department regarding the methods employed for the general bacteriological examinations of suspected cases of diphtheria, that it has been thought desirable to include in this report a condensed account of the new facts brought out in the bacteriological investigations on this subject, and together with a description of those characteristics of the diphtheria bacilli which must be known in making bacteriological examinations for diagnostic purposes.

It is hoped that with these additions this report may be of greater practical assistance to many who have begun or are about to begin similar work.

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The Facts Developed in Successive Investigations, which have led to the belief that the Bacillus first described by Klebs and Loeffler is the Specific Cause of Diphtheria.

In the year 1883, bacilli, which were very peculiar and striking in appearance, were shown by Klebs[1] to be of constant occurrence in the pseudo-membranes from the throats of those dying of true epidemic diphtheria. One year later Loeffler[2] published the results of a very thorough and extensive series of investigations on this subject. He found the bacillus described by Klebs in most, but not in all, cases of throat inflammations which had been diagnosticated as diphtheria. He separated these bacilli from the other bacteria present, and obtained them in pure culture. When he inoculated these bacilli upon the abraded mucous membrane of susceptible animals, pseudo-membranes were produced, and frequently death followed. If a certain amount of a bouillon culture was injected subcutaneously into guinea pigs, death was caused with characteristic lesions. Loeffler's failure to find the bacilli in every case examined is now explained by the fact that certain varieties of pseudo-membranous inflammation, such as occur especially in scarlet fever, were then wrongly considered to be true diphtheria.

In 1887[3], further studies by Loeffler added to the proof of the dependence of diphtheria upon the diphtheria bacilli. In 1888, D'Espine found the bacilli in 14 cases of characteristic diphtheria, and proved them to be absent in 24 cases of mild sore throats which, clinically, were believed not to be cases of diphtheria. In this same year, the first portion of the report of the results of the very important investigations of Roux[4] and Yersin was published, and the dependence of diphtheria upon the diphtheria bacilli may be considered to have been established. Roux and Yersin found that the diphtheria bacilli were present in all characteristic cases of diphtheria, and that these bacilli possessed the same cultural and pathogenic qualities as those described by Loeffler. They found that when the bacilli were inoculated upon the healthy mucous membrane of the trachea of the rabbit, no result followed; but, if the inoculation was made on the abraded membrane, phenomena occurred which strikingly resembled those present in membranous laryngitis in man, *i.e.*, congestion of the mucous membrane followed by the formation of a pseudo-membrane, œdematous swelling of the tissues, and of the glands of the neck, dyspnœa, stridulous breathing, and asphyxia. Injections of cultures beneath the skin of rabbits and guinea pigs in sufficient quantity caused their death in from thirty-six hours to five days, the period varying according to the susceptibility of the animal, and the number and the virulence of the bacteria introduced. The same results followed the injections of filtered cultures, showing that it was the products formed by the bacilli which caused the general lesions.

Roux and Yersin were also able to produce in animals characteristic diphtheritic paralysis. They effected this in many cases where the inoculated animals did not succumb to a too rapid intoxication. Paralysis commenced in a pigeon three weeks after the inoculation of the pharynx, and after all membrane had disappeared and the animal seemed to have completely recovered. In rabbits the paralysis usually commenced in

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the posterior extremities, and then gradually extended to the whole body, causing death by paralysis of the heart or respiration. In rare instances the muscles of the neck of the larynx were first paralyzed, and thus characteristic symptoms were caused. The authors conclude, "The "occurrence of these paralyses, following the introduction of the bacilli "of Klebs and Loeffler, completes the resemblance of the experimental "disease to the natural malady, and establishes with certainty the specific "rule of this bacillus."

Finally, the microscopical changes in the internal organs of animals dying of experimental diphtheria produced by the bacilli, have been shown by Welch and Flexner,[13] and by Babes[14] and others, to be essentially the same as those produced by diphtheria in man, and thus a still further proof is afforded of the specific *rôle* of this bacillus.

The results of the various observations detailed above have since been confirmed by a great number of combined clinical and bacteriological investigations, so that now all who have studied the bacteriology of diphtheria would agree with the following statement made by Welch[15] in an address on diphtheria, viz., "All the conditions have been fulfilled for "diphtheria which are necessary to the most rigid proof of the depend- "ence of an infective disease upon a given micro-organism, viz., the "constant presence of this organism in the lesions of the disease, the "isolation of the organism in pure culture, the reproduction of the "disease by inoculations of pure cultures, and similar distribution of the "organism in the experimental and in the natural disease. To be in "accord with these facts we must agree with Prudden[6] that we are now "justified in saying that the name diphtheria, or at least primary diph- "theria, should be applied, and exclusively applied, to that acute infectious "disease usually associated with a pseudo-membranous affection of the "mucous membrane, which is primarily caused by the bacillus called "the bacillus diphtheriæ of Loeffler."

Pseudo or False Diphtheria.

Under this general title are included all cases of pseudo-membranous or exudative inflammation of the mucous membranes in which the diphtheria bacillus is absent.

As has already been stated the thorough consideration of the bacteriology of this form of inflammation is to be reserved for a later report, but it is necessary to touch on a few points here.

Since Loeffler,[2] in 1889, first described a class of pseudo-membranous inflammations of the throat in which the diphtheria bacilli were absent and cocci present, it has been established that a certain proportion of the inflammations of the respiratory mucous membranes, which closely resemble the less characteristic cases of diphtheria, are not due to the diphtheria bacilli but to cocci, especially to streptococci.

It has been found that streptococci are regularly present in the throat, at least in the throats of persons living in large cities, and that other forms of cocci, especially the pneumococci and staphylococci, are apt to be associated with them. These germs seem to live in the throat without creating any disturbance so long as the mucous membranes are healthy,

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but under certain conditions when the mucous membrane has been injured by exposure to cold, or by the poison of scarlet fever, measles, or some other disease, the streptococci, alone or associated with other cocci, are able to attack the mucous membrane, and to cause an inflammation. This may be of any degree of intensity, from a simple inflammatory hyperæmia to an inflammation with the extensive production of pseudo-membrane or with ulceration. Such inflammations when associated with the formation of a pseudo-membrane are known as pseudo-diphtheria. The exudate or pseudo-membrane in pseudo-diphtheria is usually confined to the tonsils, but other portions such as the larynx, pharynx, and nostrils may be invaded.

It has been found that the percentage of mortality in these cases is far less than in diphtheria, and that the disease is seldom if ever communicated to others.

The Proportion of Cases of Suspected Diphtheria which upon Examination prove to be True Diphtheria.

As soon as careful investigation had demonstrated that it was possible, with proper precautions, to separate by bacteriological examination the cases of true from the cases of false diphtheria, large numbers of cases suspected to be diphtheria were examined bacteriologically. The reports from those hospitals in which all cases of diphtheria and of suspected diphtheria were examined are of especial interest as showing the proportion of cases of true to false diphtheria. The results from these hospitals are all the more valuable because the cases came from all parts of the various cities in which the respective hospitals were located, and hence special local conditions were not likely to greatly influence the general results obtained. Thus Baginsky,[7] in Berlin, found the diphtheria bacilli in 120 out of 154 cases; Martin,[8] in Paris, in 126 out of 200; Janson,[9] in Switzerland, in 63 out of 100; Morse,[10] in Boston, in 239 out of 400; and Park,[11] in New York, in 127 out of 244. Thus from 20 to 50 per cent. of the cases sent to diphtheria hospitals did not have diphtheria.

If we examine the reports of examinations made under some special conditions, as during an outbreak of some contagious disease in a children's hospital, we find the results may differ in a striking manner.

Thus, in 1889, Prudden[12] made bacteriological examinations of 24 fatal cases of pseudo-membranous inflammation of the tonsils, pharynx, and larynx. In none of these were the Loeffler bacilli found to be present. These cases occurred in two children's hospitals in New York, in which both scarlet fever and measles were at the time prevalent. During the past year we have examined the exudate from 46 fatal cases of suspected diphtheria occurring in these same institutions, and found the bacilli present in 44 of the same.

If scarlet fever and measles (but not true diphtheria) were prevailing in an institution, it is evident that the bacilli would be absent from the pseudo-membranes occasionally, occurring in the throat as a complication of these diseases.

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The Mortality in True Diphtheria and in Pseudo-Diphtheria.

All observers have found that the mortality was far higher in those cases in which the diphtheria bacilli were present than in those in which they were absent. In true diphtheria the mortality has been found to vary from 25 per cent. to 70 per cent, while in pseudo-diphtheria it varies from 0 per cent. to 20 per cent.

The death rate in cases of pseudo-diphtheria occurring in hospitals averages far higher than the death rate in similar cases in the cities at large. The reason for this is chiefly to be found in the fact that quite a large proportion of such cases admitted to the hospitals have been removed there either because they were suffering from laryngeal obstruction or because the throat affection was complicated by some infectious disease. Nearly all fatal cases of pseudo-diphtheria belong to one or the other of these classes.

Collection of the Blood Serum, and its Preparation for Use in Cultures.

A covered glass jar which has been thoroughly cleansed with hot water is taken to the slaughter-house and filled with freshly-shed blood from a calf or sheep. The blood is received directly in the jar as it spurts from the cut in the throat of the animal. After wiping the edge of the jar, it is covered with the lid and set aside where it may stand quietly until the blood has thoroughly clotted. The jar is then carried to the laboratory and placed in an ice chest. If the jar containing the blood is carried about before the latter has clotted, very imperfect separation of the serum will take place. It is well to inspect the blood in the jar after it has been standing a few hours, and, if the clot is found adhering to the sides, to separate it by a rod. The blood having remained twenty-four hours on the ice, the serum which surrounds the clot is syphoned off by a rubber tube and mixed with one-third its quantity of nutrient beef broth, to which one per cent. glucose has been added. The broth is prepared as follows :—One pound of finely-chopped lean beef is allowed to soak in one litre of water in a cool place for at least twelve hours. The meat and fluid are now dumped into a cheese-cloth or towel, and the fluid squeezed out. To this solution one per cent. peptone, one per cent. of glucose, and one-half per cent. of common salt are added. It is well to test the reaction of the mixture, and, if it is found to be acid, to render it neutral by adding a few drops of a solution of caustic or carbonate of soda. The whole is now boiled for half an hour, and filtered through absorbent cotton or filter paper. If the broth is to be kept it should be placed in flasks and then sterilized. The Loeffler blood serum mixture when ready is poured into tubes, which should be about four inches in length, and two-thirds of an inch in diameter. These tubes should first be plugged with cotton, and sterilized by dry heat at 150° C. for one hour. Care should be taken in filling the tubes to avoid the formation of air bubbles, as they leave a permanently uneven surface when the serum has been coagulated by heat. To prevent this, the end of the pipette or funnel which contains the serum should be inserted well into the test tube. About two centimètres

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are sufficient to each tube. The tubes, having been filled, are now to be coagulated and sterilized. The tubes are placed at the proper angle, and then kept for two hours at a temperature just below the boiling point. For this purpose a Koch serum coagulator or a double boiler serves heat, though a steam sterilizer will suffice. If the latter is used, a wire frame must be arranged to hold the tubes at the proper inclination, and the degree of heat must be carefully watched, as otherwise the temperature may go too high, the serum actually boiled, and the culture medium thus spoiled. After sterilization by this process the tubes containing the sterile, solidified blood serum can be placed in covered tin boxes and kept for months. The serum prepared in this way is quite opaque and firm. A mixture of blood cells renders the serum darker, but it is not less useful.

The Swab for Inoculating Culture Tubes.

The swab to inoculate the serum is made as follows: A stiff, thin steel iron rod, six inches in length, is roughened at one end by a few blows of a hammer, and about this end a little absorbent cotton is firmly wound. Each swab is then placed in a separate glass tube, and the mouths of the tubes are plugged with cotton. The tubes and rods are then sterilized by dry heat at about 150° C. for one hour, and stored for future use. These cotton swabs have proved much more serviceable for making the inoculation than platinum wire needles, especially in young children and in laryngeal cases. It is easier to use the cotton swab in these cases, and it gathers up so much more material for the inoculation that it has seemed more reliable.

For convenience and safety in transportation "a culture outfit" has been devised which consists of a small wooden box containing a tube of blood serum, a tube holding a swab, and a record blank. These "culture outfits" may be carried or sent by messenger or express to any place desired, and are kept at stations scattered throughout the city for the free use of physicians.

Directions given Physicians and Department Inspectors for Inoculating the Tubes with the Exudate.

The patient is to be placed, when possible, in a good light, and, if a child, properly held. Taking the swab from its tube, the tongue is depressed with a spoon, and the swab is passed as clear as possible of the tongue, and rubbed gently but firmly against any visible membrane on the tonsils or pharynx, thus catching the exudate with its contained bacteria in its meshes. Immediately, without laying it down, it is inserted in the blood serum tube, and the portion of the swab which had previously been in contact with the exudate is rubbed a number of times back and forth over the whole surface of the serum. This, though thoroughly, is to be gently done, so as not to break the surface of the serum. Both tubes, their cotton plugs having been replaced, are put in the box and sent to the collecting station. From here they are taken by the collector to the laboratory and placed in the incubator, and there left for from twelve to fourteen hours, after which time they are ready for examination.

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Where there is no visible membrane, but it is thought to be present in the nose or pharynx, the swab should be thoroughly rubbed over the mucous membrane of the pharynx and tonsils. In nasal cases, when possible, a culture should also be made from the nose. In little children, care must be taken not to use the swab when their throats contain food or vomited matter, as when this happens the bacterial examination is rendered more difficult. Under no conditions should inoculations be made shortly after the application of disinfectants (especially solutions of corrosive sublimate) to the throat. If any of these instructions have not been carried out, the fact should be carefully noted on the record blanks.

The Examination of Cultures.

The culture tubes which have been thus inoculated are kept in an incubator at 37° Cent. for twelve hours, and are then ready for examination. On inspection it will be seen that the surface of the blood serum is dotted with very numerous, just visible, colonies. At this time no diagnosis can be made from simple inspection. If, however, the serum is found liquefied, or shows other evidences of contamination, the examination will probably be unsatisfactory. A microscopical preparation is now prepared by placing a tiny drop of water upon a clean cover glass, and then a platinum needle is inserted in the tube, and quite a large number of colonies are swept from the surface of the culture medium. The bacteria adherent to the needle are washed off in the drop of water on the cover glass, smeared over its surface and dried in the air. The cover glass is then passed quickly through the flame of a Bunsen burner or alcohol lamp three times in the usual way, covered with a few drops of Loeffler's solution of alkaline methyl blue, and left without heating for ten minutes. The cover glass is then rinsed off in clean water, dried and mounted in balsam.

In the great majority of cases one of two pictures will be seen with the one-twelfth oil immersion lens: either an enormous number of characteristic Loeffler bacilli with a moderate number of cocci, or a pure culture of cocci mostly in pairs or short chains. In a few cases there will be an approximately even mixture of Loeffler bacilli and cocci, and in others a great excess of cocci. Besides these will be met occasionally preparations in which, with the cocci, there are bacilli resembling more or less the Loeffler bacilli. These bacilli, which belong to the pseudo-diphtheria bacilli, are especially frequent in cultures from the nose.

In not more than one case in twenty will there be any serious difficulty in making the diagnosis if the serum tube has been properly inoculated. In such a case another culture must be made.

An immediate diagnosis, without the use of cultures, is often possible. This is made by smearing a cover glass with a little exudate from the swab, drying, staining, and examining it microscopically. This examination, however, is much more difficult and the results more uncertain than when the covers are prepared from cultures. The bacilli from the membrane are usually less typical in appearance than those found in the cultures, and they are mixed with fibrin, pus, and epithelial cells; they

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may also be very scanty in the parts reached by the swab, or we may meet with bacilli which closely resemble the Loeffler bacilli in appearance, but which differ greatly in growth. When in a smear containing mostly cocci a few of these doubtful bacilli are present, we are unable to certainly exclude the diagnosis of diphtheria. Although in certain cases this immediate examination may be of the greatest value, it is not a method suitable for general use.

The Diphtheria Bacillus.

When cover glass preparations made from the blood serum tubes are examined, the diphtheria bacilli are found to possess the following characteristics :—

The diameter of the bacilli varies from 0·3 to 0·8 millimètre and the length from 1·5 to 6·5 millimètres. They occur singly and in pairs and very infrequently in chains of three or four. The rods are straight or slightly curved, and usually are not uniformly cylindrical throughout their entire length, but are swollen at the ends, or pointed at the ends and swollen in the middle portion. Even from the same culture the different bacilli differ greatly in their size and shape. The two bacilli of a pair may lie with their long diameter in the same axis, or at an obtuse or an acute angle. The bacilli possess no spores, but have in them highly refractile bodies. They stain readily with the ordinary aniline dyes, and retain their colour after staining by Gram's method; with an alkaline solution of methyl blue the bacilli, from blood serum cultures especially, and from other media less constantly, stain in an irregular and extremely characteristic way. The bacilli do not stain uniformly; in their ends, or in their central portions, certain oval bodies stain much more intensely than the rest of the bacillus. Sometimes this highly stained body is thicker than the rest of the bacillus, again it is thinner, and surrounded by a more slightly stained portion. The bacilli seem to stain in this peculiar way at a certain period in their growth, so that only a portion of the bacilli taken from a culture at any one time will show the characteristic staining. In old cultures the bacilli frequently stain with great difficulty, and often not at all characteristically.

Growth on Blood Serum.

If we examine the growth of the diphtheria bacillus in pure culture on blood serum we shall find at the end of ten to twelve hours little colonies of bacilli, which appear as pearl-grey or whitish-grey slightly raised points. The colonies when separated from each other increase in forty-eight hours to a diameter of a quarter of an inch. Their borders are usually somewhat uneven. Those lying together will fuse into one mass, especially if the serum is rather moist. During the first twelve hours the colonies of the diphtheria bacilli about equal in size those of the streptococci, but after this time the diphtheria colonies become larger than those of the streptococci, nearly equalling those of the staphylococci. The diphtheria bacilli in their growth never liquefy the blood serum.

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Growth of One per cent. Alkaline Glycerine Agar.

It is frequently desired to obtain the diphtheria bacilli in pure culture. This is most readily accomplished by removing with a platinum needle a portion of the mixed growth of bacteria in a serum tube, and lightly streaking it over the surface of the nutrient agar contained in a Petri dish.[1]

Though the growth of the diphtheria bacilli upon agar is less certain and luxuriant than upon serum, the appearance of the colonies when examined under the microscope is more characteristic.

If the diphtheria colonies develop deep in the substance of the agar, they are usually round or oval, and, as a rule present no extensions; but if near the surface, usually from one, but sometimes from both sides, they will spread out in an apron-like extension, which exceeds in extent the rest of the colony. When the colonies develop entirely on the surface, they are more or less coarsely granular, are nearly translucent, and usually have a darker centre. The edges are sometimes jagged, and frequently shade off into a delicate lace-like fringe; at other times the margins are more even, and the colonies are nearly circular. With a high-power lens the edges show the sprouting bacilli. The colonies are greyish or greyish-white by reflected light, and pure grey or with olive tint by transmitted light.

The growth of the diphtheria bacillus upon agar presents certain peculiarities which are of the utmost practical importance. While the bacilli from the majority of cases grow rather feebly, some grow luxuriantly. If a large number of the bacilli from a recent culture are implanted upon a properly prepared agar plate, a certain and fairly vigorous growth will always take place. If, however, the agar is inoculated with the exudate of a throat which contains but few Loeffler bacilli, no growth whatever of the bacilli may take place; while the tubes of coagulated blood serum inoculated with the same exudate contain them abundantly. Again, agar prepared from broth made from different specimens of beef, or to which different peptones have been added, varies somewhat as to its suitability for the growth of the bacilli. On account of this uncertainty of obtaining a growth by the inoculation of agar with a few bacilli, or with bacilli of diminished vigour, agar is a far less reliable material than blood serum for making cultures for diagnostic purposes, and is, therefore, not to be recommended. All agar should be tested by means of a pure culture of the diphtheria bacillus before being used experimentally.

Growth in Broth.

All the varieties of the Loeffler bacillus experimented with have grown in slightly alkaline broth, with or without the addition of one per cent. glucose. The characteristic growth is one in very fine grains. These

[1] The agar is prepared by adding one per cent of agar to the required quantity of broth. This broth is prepared in the same way as that used in the blood serum mixture already described, except that it contains no glucose. The agar must be thoroughly dissolved in the broth, and to accomplish this it is necessary to boil the mixture for from three to six hours. Before filtering, sufficient alkali must be added to make the agar slightly but distinctly alkaline. Finally, six per cent. of glycerine is added and the mixture sterilized in flasks. When needed, it is melted and poured into sterilized Petri dishes in a thin layer.

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grains deposit along the sides and bottom of the tube, leaving the broth nearly clear. In some, for twenty-four to forty-eight hours, there is a more or less diffuse cloudiness, and exceptionally a film forms over the surface of the broth. Upon shaking the tube, this film breaks up and slowly sinks to the bottom. All the varieties tested caused the alkaline broth to become acid, or, at least, distinctly less alkaline, within forty-eight hours.

Animal Inoculation as a Test of Virulence.

Animal experiments form the only reliable method of determining with absolute certainty the virulence of the diphtheria bacillus. For this purpose alkaline glucose bouillon cultures of forty-eight hours' growth were used for the subcutaneous inoculation of guinea pigs. The amount injected varied from a quarter to a half per cent. of their body weight. In the great majority of cases this amount caused death within seventy-two hours. Upon autopsy the characteristic lesions described by Loeffler are found, namely: at the seat of inoculation there is a greyish focus surrounded by an area of congestion; the subcutaneous tissues for an extensive area around are congested, and at times very œdematous, and at times but slightly so; the adjacent lymph nodes are swollen, and the serous cavities, especially the pleural, frequently contain an excess of fluid, usually clear, but at times turbid; the lungs are usually congested. If the organs are subjected to microscopical examinations the lesions, as described by Welch and Flexner,[13] Babes[14] and others, are found. There are numerous smaller and larger masses of necrotic cells, which are permeated by leucocytes. The heart and voluntary muscle fibres usually show degenerative changes. The number of leucocytes is increased in the blood. From the area surrounding the point of injection virulent bacilli may be obtained, but in distant areas and organs they are only occasionally found.

Bacilli, which in cultures and in animals have shown themselves to be characteristic, may be regarded as certainly true diphtheria bacilli, and as capable of producing diphtheria in man under favourable conditions.

A large portion of the daily work in the laboratory has consisted in the routine examination of the cultures received each day. Besides this, however, a number of important questions have been studied experimentally, of which the most important are the following:—

1. How much reliance can be placed upon the bacteriological diagnosis made from the examination of a culture inoculated with the throat secretion from a case of suspected diphtheria?

2. If there are found in cultures bacilli which possess the shape, size, and staining characteristics of the diphtheria bacillus, can they, without further cultural or animal experiments, be considered as virulent diphtheria bacilli, and if not, what is the liability of error?

3. What is the period of time during which virulent diphtheria bacilli remain in the throat after the disappearance of the exudate or pseudo-membrane?

4. What relation have the pseudo and the non-virulent diphtheria bacillus to the true virulent bacillus?

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5. To what degree is pseudo-diphtheria communicable?
6. What are the means by which diphtheria is transmitted?

Results of Original Investigations. The Amount of Dependence which can be placed upon a Culture in making a Diagnosis.

During the first few months, in order to test the results of the examinations and to make the liability to error as slight as possible, the following plan was adopted :—

All cases which yielded no diphtheria bacilli were turned over to special inspectors, who made in every case, where possible, a second culture, and followed up the case for some time, even after its recovery.

By means of this check the bacteriologists of the Department were able more and more surely to decide how far they could base an absolute diagnosis upon a culture, especially one made by others. Many physicians, as well as the inspectors, gradually became so skilled that it was possible to rely certainly upon their results, while, on the other hand, caution was found to be necessary in accepting the inoculations of others, and here a second culture was requested.

After a year's trial, the following conclusions have been arrived at :— The examination by a competent bacteriologist of the bacterial growth in a blood serum tube which has been properly inoculated and kept for fourteen hours at the body temperature can be thoroughly relied upon in cases where there is visible membrane in the throat, if the culture is made during the period in which the membrane is forming, and no antiseptic, especially no mercurial solution, has lately been applied.

In cases in which the disease is confined to the larynx or bronchi, and where, therefore, there is no visible exudate against which the swab can be rubbed, surprisingly accurate results can be obtained from cultures, but in a certain proportion of cases no diphtheria bacilli will be found in the first culture, and yet will be abundantly present in the later ones. We believe, therefore, that absolute reliance for a diagnosis cannot be placed upon a single culture from the pharynx in purely laryngeal cases. The apparent mistakes have, however, been few. In nasal diphtheria it is possible to obtain negative results from a culture made from the throat, and yet the bacilli be found in cultures from the nose. In making a diagnosis from a culture it is essential to know the duration of the disease in the case from which it was made, for, although bacilli may remain present and alive in some throats for a surprising length of time, nevertheless, it is important to remember that they may vanish early and suddenly, and that, therefore, the cultures cannot be certainly relied upon after the membrane begins to disappear.

The use of antiseptics shortly before making the inoculation of the tube may render the culture useless for diagnosis. It has been found in a few instances that a culture made from a case of diphtheria shortly after a thorough irrigation with a 1-4000 solution of bichloride of mercury gave no diphtheria bacilli, though one made just before and one made some time later gave them abundantly. A curious fact is that under such circumstances a vigorous growth of other organisms may take place.

The above conclusions are true only when the inoculations have been

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properly made, and in judging cultures received from physicians in general the greatest care must be taken. Some are made carelessly, and some evidently without taking the pains to even read the instructions, or to glance at the condition of the coagulated serum in the tube. If, therefore, when no diphtheria bacilli are found, the bacterial growth is scanty, the media dry or contaminated, or the inoculation in any way faulty, the case must be referred back for another culture. The second culture in these cases not infrequently contains the bacilli where the first did not.

The absence of the bacilli in a culture proves the case to be one of false diphtheria only when it has been possible to make it under the proper conditions.

The Virulence of Bacilli in Cases of suspected Diphtheria.

Since it is the custom in the laboratory of the Health Department to make a bacteriological diagnosis in suspected cases of diphtheria, from the examination of the growth upon the original blood serum tube without waiting for further cultural or animal experiments, it is of the greatest practical importance to ascertain to what extent bacilli appearing upon the serum in every way characteristic of the diphtheria bacilli can be assumed to be virulent.

To test the virulence of bacilli, it is necessary to obtain them in pure culture, for otherwise it would be impossible to determine whether the changes produced in the inoculated animal were due to the supposed diphtheria bacilli or to other micro-organisms injected with it. It is still further necessary to grow the bacilli in proper media, and to inoculate a susceptible animal at the period when the growth of the bacilli in the media has reached its maximum. It is only when these precautions have been followed that accurate and positive results will be obtained. The present almost uniform practice is to inoculate half-grown guinea-pigs with from a quarter to a half per cent. of their body weight of a forty-eight hours' culture of the bacilli grown at 37° Cent. in nutrient or glucose alkaline broth. It is not safe to decide that because the growth derived from one bacillus is not virulent, all the bacilli from that throat are not virulent. The cultures from several bacilli must be tried. The majority of those who have inoculated bacilli derived from pseudo-membranes, and possessing the characteristics of the Loeffler bacilli found, as Loeffler did, that they were always virulent. The researches of Hofmann,[15] Beck,[16] and others, showing that in a certain number of healthy throats there were bacilli which closely resembled the Loeffler bacillus and yet were not virulent, stimulated others to subject the bacilli from large numbers of cases of suspected diphtheria to the test of animal inoculation.

In 1890, Roux and Yersin[4] published the results of some examinations as to the virulence of the bacilli obtained from one hundred cases of diphtheria. Fifty-five of these were fatal cases, and in all of them virulent bacilli were found, although in a few they found, together with many virulent bacilli, a few non-virulent ones. Among the forty-five cases which recovered, many were very mild, and in ten of them they

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found no bacilli of sufficient virulence to cause the death of a guinea pig, when injected in a moderate amount. From all of them, however, they obtained bacilli capable of causing inflammation in the guinea-pig at the point of injection. This varied from extensive necrosis to slight, transient œdema. From further experiments, they proved that similar bacilli were capable, under proper conditions, of regaining their virulence. They further showed that in these milder cases, among many non-virulent or slightly virulent bacilli, there were usually a few virulent ones; therefore they believe that in most of these ten cases fully virulent bacilli may have been present in the throat with the slightly virulent ones, which by chance were used for the inoculations. In similar investigations, carried on in a different locality, somewhat different results have been obtained. Escherich [7] was unable to obtain from a large number of diphtheria cases studied any bacilli having the characteristics of the Loeffler bacillus which were not virulent, and only a few which, in injections of a quarter per cent. of the body weight of a forty-eight hour bouillon culture, did not kill guinea-pigs within forty-eight hours. Koplik, in testing the virulence of bacilli from mild cases of tonsillar diphtheria, found them in every case fully virulent.

Prudden, in eleven cases of diphtheria, and Welch and Abbott in eight, found the bacilli in every case fully virulent. In a later investigation, in which a large number of healthy and slightly inflamed throats were examined, Abbott found, in the cultures from three, bacilli resembling the Klebs-Loeffler bacilli, but lacking virulence. These will be considered bacteriologically in connection with the pseudo-diphtheria bacilli, but the cases are of interest in the present consideration of the virulence of bacilli obtained from throats in which inflammatory lesions have appeared, which more or less resemble diphtheria.

Case 1: Adult, aged fifty-nine. While in hospital developed a laryngitis and pharyngitis. The uvula, tonsils, and faucial pillars became swollen and œdematous, of an intense crimson-red colour, and covered with a thin, greyish-white, slightly adherent exudate. In five days the patient completely recovered. Bacteriological examination showed abundant, apparently characteristic, diphtheria bacilli, which, when inoculated, proved not to be virulent.

Case 2: Similar lesions to last; well on ninth day. Bacteriological examination—abundant bacilli, in appearance similar to Klebs-Loeffler bacillus, but not virulent.

Case 3: Girl, aged eleven years. Acute tonsillitis, with small white plug in one crypt. Quick recovery. Bacteriological examination—apparently characteristic Loeffler bacilli, but not virulent.

It must remain a matter of doubt whether some colonies from these cases would have been found to possess virulence if more had been tested as to this characteristic. These cases, as well as those of Roux and Yersin, show that now and then the bacilli from suspicious cases have little or no virulence.

In order to determine the virulence of the bacilli obtained in the ordinary routine examinations from suspected cases of diphtheria, blood serum cultures from 25 cases were selected, in which bacilli were found

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in appearance characteristic of the virulent diphtheria bacilli. Nineteen of these comprised the total number of cultures containing Loeffler bacilli received during one day. These cultures were selected without knowledge of the severity of the case from which they were obtained, and were used for experiments on animals. From twenty of these, the Loeffler bacillus was isolated in pure culture, and inoculated into guinea pigs with results as stated in the following table. From the remaining five it was found impracticable to obtain the Loeffler bacillus in pure cultures, on account of the presence in relatively large number of other more rapidly growing organisms. Experimental work on these was therefore discontinued.

Virulence of the Bacilli found in Twenty Cases of Throat Inflammations of such a character as to arouse a suspicion of the existence of Diphtheria.

	Severity.	Weight of Guinea Pig. Gms.	Amount of culture injected. c.c.	Duration of life after inoculation.	Persistence of Loeffler bacillus after recovery of patient.
1	Very mild case; sick only 4 or 5 days	485	2	40 hours	14-19 days
2	Subsequently contracted scarlet fever	305	1	12 days	*Note.* — Pig previously inoculated with non-virulent culture.
3	Mild case.....	350	1	45 hours	24-32 days
4	Mild case.....	900	3	40 hours	
5	Diag. — Char. follicular tonsillitis with history of exposure to diphtheria	405	1	40 hours	6 days
6	Very mild case: culture taken after disappearance of membrane	430	1·5	40 hours	13 days
7	Very mild case.....	410	1·5	40 hours	
8	Fatal case, and cause of severe case in mother	435	1·33	40 hours	P. 16 days
9	Mild case.....	390	1·33	40 hours	P. 38 41 days
10	Mild case; adult; never in bed	210	0·5	50 hours	P. 44 days
11	Removed to diphtheria hospital; severe case	220	0·5	40 hours	
12	Rather mild case.....	620	3·33	25 hours	P. 42 days
13	Very mild case.....	479	2	40 hours	P. 20-24 days
14	Fatal case; croup	675	1·5	40 hours	
15	Fairly severe case, followed by measles	443	1·33	40 hours	P. 15-23 days
16	Moderately severe case ...	435	1·33	4 days	P. 15 19 days
17	Moderately severe case ...	510	1·66	40 hours	
18	Fatal case; croup	475	1·5	40 hours	
19	Very mild case.....	500	1·66	40 hours	
20	Contracted from a mild case; no membrane present	250	1	40 hours	

We find, therefore, that the bacilli obtained from twenty cases of suspected diphtheria, two-thirds of which were very mild cases, proved

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in every case to be virulent, and in all but three fully so. If these results are considered in connection with those obtained by Koplik and Prudden in New York, as well as those obtained by other American and European observers, it is possible, without making animal inoculation, to state when, in a case of throat inflammation suspected to be diphtheria, bacilli are found in the cultures possessing the morphological and cultural characteristics of the Loeffler bacilli that the bacilli are in all probability virulent, viz., they are capable under the proper conditions of causing diphtheria in others. Further, it should be stated, as shown by Roux and Yersin, and as confirmed by others and by ourselves, that the absence of virulence in a culture made from one bacillus is not sufficient to prove that cultures from other bacilli from the same case would not be virulent.

In three of the above cases the cultures from the first colony selected were not virulent, while from others they were fully so.

The Length of Time during which the Diphtheria Bacillus Persists in the Throat after the Disappearance of the Membrane, and its Virulence in these Convalescent Cases.

If a piece of membrane is removed from a throat during the period of invasion of diphtheria, and examined microscopically or by cultures, the presence of abundant diphtheria bacilli will be noted. If, a few days later, when the membrane has begun to loosen, another bit be examined the diphtheria bacilli will be found to be partly or, at times, wholly replaced by other micro-organisms, mostly cocci. If, several days after the disappearance of the membrane, cultures be made from the mucus of the throat, it will be found that the bacilli of diphtheria in a majority of cases will have disappeared from the throat. This rule is not, however, without many exceptions, for it will be frequently found that days after the complete disappearance of the membrane, and after the return of the throat to a healthy condition, fully virulent bacilli linger in the throat.

If we examine the researches of others we find the following facts :—

	Severity of Disease.	Length of time during which the virulent bacilli persisted after the disappearance of the exudate.	Results obtained from the Inoculation of Guinea Pigs.
	ROUX & YERSIN :—		
1	Mild case	3 days	Guinea pig died—24 hours.
2	Mild case	3 days	Fully virulent. Killed in a few hours.
3	Severe laryngeal case	11 days	Guinea pig died in 3 days.
4	Severe case	14 days	Guinea pig. Fully virulent.
5	Mild case	9 days	Some colonies virulent, some not virulent. For one week more, only non-virulent colonies.
6	Mild case	7 days	Virulent and non-virulent colonies. For 4 days more only non-virulent found.

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	Severity of Disease.	Length of time during which the virulent bacilli persisted after the disappearance of the exudate.	Results obtained from the Inoculation of Guinea Pigs.
	ROUX & YERSIN :—		
7	Laryngeal case.....	15 days	On 12th day, all virulent. On 15th, some not virulent.
8	Severe case	2 months	Produced a slight local œdema only when injected into guinea pigs.
	KOPLIK :—		
9	Mild case	14 days	Fully virulent.
10	Mild case	7 days	Virulent : a week later the bacilli obtained were not virulent.
	LOEFFLER :—		
11	Moderate case	8 days	Fully virulent (this was twenty-fourth day of disease).

ESCHERICH :—In a number of cases the Loeffler bacilli were found to persist after the disappearance of the membrane. In all of these the bacilli were as virulent as those obtained at the height of the disease.

MORSE found in twenty-five cases that the average length of time the Loeffler bacillus remained in the throat after the disappearance of the membrane was ten days.

The average duration was the same for both nose and throat, although in some cases the bacilli were found in the throat much longer than in the nose, and *vice versâ*. The bacilli disappeared in one case the day after the throat was clear, in another three days after, and in another four days after. The longest periods during which they persisted were twenty-two and thirty-seven days. The bacilli were tested from only one case, and these were fully virulent ten days after the disappearance of the membrane from the throat.

TOBIESEN found virulent diphtheria bacilli in the throats of twenty-four out of forty-six patients at the time of their discharge from the hospital. The majority were children between six and twelve years. The following table gives the length of time after convalescence that the diphtheria bacilli were found :—

Severity of Case.	Number of Cases.	Duration of bacilli after disappearance of membrane.
Mild	1	4
Moderate	5	4
,, ...	4	5
,, ...	4	6
Mild	1	8
Moderate	1	8
,, ...	1	9
Severe	1	9
Moderate	1	10
Mild	1	10
Moderate	1	11
Severe	1	16
Moderate	1	22
,, ...	1	31
	24	

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In the twenty-two in which the bacilli were not found the length of stay in hospital of the patients after convalescence was about the same. His studies indicated that the existence of throat lesions rendered the conditions more favourable for the persistence of bacilli. The virulence of the bacilli was proven in nineteen out of the twenty-four. In sixteen cases the guinea pigs died within a period of fifty hours, and the autopsies showed typical lesions; in two local necrosis developed, followed by death in one animal and recovery in the other. In the last case the animal developed paralysis five weeks after the local symptoms had disappeared. From these results he draws the following conclusions :—

In nineteen out of the twenty-four persistent cases the Loeffler bacilli proved virulent, and the probability is that they were also virulent in the five not tested. In other words, one-half of the patients who are allowed to leave the hospital under the usual conditions carry virulent bacilli in their throats, and are capable of giving diphtheria to others. Clinical investigation alone can decide the frequency with which these convalescent cases infect others. This must be carried on with great caution. In the twenty-four investigated by Tobiesen he excluded those where numerous cases had occurred in the house besides the ones under investigation. Among those remaining he discovered one where the convalescent child was the almost certain cause of diphtheria in another.

In order to test the virulence of the bacilli in the throats of convalescent cases, they were obtained in pure culture from the healthy throats of thirteen convalescent diphtheria cases, and used for the inoculation of guinea pigs. The following table gives the results of these experiments.

Case No.	Severity.	Bacilli persistent after recovery for	Virulence.			Persistence from inception of disease.
			Weight guinea pig gms.	Amount injected.	Life of guinea pig after injection.	
1 (1300)	App. severe case but very quick recovery	8 days	392	1·33 c.c.	60-70 hrs.	13-17 days
2 (527)	Mild case	10 days	250	0·5 c.c.	8 days	12-19 days
3 (1358)	,, ...	12 days	290	1·25 c.c.	11 days	(?)
4 (956)	Severe case.....	18 days	229	1 c.c.	9 days	21-30 days
5 (685)	Moderate case..	6 days	549	1·25 c.c.	14 days	10-22 days
6 (909)	Mild case	33 days	226	1 c.c.	—	
			(Extensive necrosis with final recovery)			
7 (1.13)	Very mild case..	12 days	440	1·5 c.c.	About 40 hrs.	14-22 days
8 (R. Weed)	Mild case	8 days	310	2 c.c.	C. 40 hrs.	16-20 days
10 (1.19)	Very mild case..	25 days	505	1·66 c.c.	C. 40 hrs.	30 (?)
11 (1.20)	Very mild case (nasal)	10 days	253	2 c.c.	C. 40 hrs.	10 (?)
12 (1.21)	Lesion. Brother had mild case	6 days	490	1·66 c.c.	C. 40 hrs.	24 (?)
13 (1442)	Mild case	8 days	450	1·33 c.c.	C. 40 hrs.	13-20 days
14 (1.23)	Recovered	12 days	367	1·33 c.c.	C. 40 hrs.	19 days
15 (1.24)	Fairly severe case	26 days	347	1·33 c.c.	C. 5 days	35-44 days

The cultures, from which the bacilli were taken in each case to test the virulence, were usually the last or next to the last in which the bacill

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were found to be present. The results in these fifteen cases, as well as those before recorded by others, prove conclusively that the bacilli, which in a certain proportion of cases persist in the throat after an attack of diphtheria, are always virulent for some time. In the exceptiona cases, in which the bacilli persist for a very long time, they are found occasionally to lose their virulence a few days before their final disappearance, while in other cases they retain their virulence to the end. That the cases themselves do not seem so liable to spread diphtheria is probably largely because of the relatively small number of bacilli present in the convalescent throats as compared with those showing the lesions of diphtheria.

During the last six months completed observations have been made in 605 cases as to the length of time during which the Loeffler bacilli persist in cases of diphtheria. In these cases cultures were made at the beginning of the disease, and then again at short intervals after the complete disappearance of the exudate, until the throat was found to be free of diphtheria bacilli. The custom was to make the second culture three days after the complete disappearance of the membrane, and then to repeat them when necessary about every fourth or fifth day. In 304 of these 605 cases the diphtheria bacilli disappeared within three days after the complete disappearance of the exudate. In 301 cases the diphtheria bacilli persisted for a longer time, viz., in 176 cases, for seven days ; in 64 cases, for twelve days ; in 36 cases, for fifteen days ; in 12 cases, for three weeks ; in 4 cases, for four weeks ; and in 4 cases, for five weeks after the time when the exudate had to all appearances completely disappeared from the upper air passages.

On account of the length of this report the bacteriology of pseudo-diphtheria will be largely left for a special report to be published later.

The Pseudo-Diphtheria Bacillus and its Relation to the Diphtheria Bacillus.

In 1888 Hofmann published the results of the bacteriological examinations of a number of diseased and healthy throats, which for a time threw doubt upon the specific character of the Loefiler diphtheria bacillus. Further research has entirely dispelled the objections which his discoveries seemed to make, but the results of these studies, and of similar ones, are of such practical importance in relation to the bacteriological diagnosis of cases of suspected diphtheria, that a detailed account of the work of others, as well as that of the Health Department, will be presented.

Hofmann confirmed Loeffler's results, in that he found the virulent bacillus in all of eight cases of true diphtheria, but in further search he was surprised to find in the throats of 26 out of 45 persons, none of whom were suffering from diphtheria, a bacillus which very closely resembled the Loeffler bacillus. Some of these persons were suffering from scarlet fever, measles, or some other disease, while many were entirely healthy. The bacilli from a number of these cases were obtained in pure culture, and inoculated into animals. The majority had no virulence whatever. The bacilli from the different cases varied somewhat in their characteristics. Some in appearance, manner of staining, and growth on media seemed identical with the Loeffler bacillus, while others pre-

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nted slight but constant differences. Between the extremes were many gradations.

Those of the bacilli which did not possess all the characteristics of the virulent bacillus differed in the following respects. The bacilli were shorter and thicker, and more uniform in size. On agar they grew in whiter and thicker colonies, whose circumference was more circular and less notched. They also grew at a lower temperature than the virulent bacilli (20°, 22° C.).

Hofmann was undecided whether all of these bacilli were really Loeffler diphtheria bacilli which had lost their virulence, or whether they were a different species of bacteria, and of a saprophytic nature. He was also undecided whether even among these non-virulent bacilli there might not be included different species. Hofmann's death prevented further attempts on his part to solve this problem, and different investigators since that time have been divided in their opinions; some taking the view that these bacilli were derived from true Loeffler bacilli, having merely lost their virulence; others, that they were a different species, having no connection with the Loeffler bacillus; and still others consider the matter as undecided. That we may be better able to consider the nature of these bacilli we will examine carefully the results of two important investigations, those of Roux and Yersin, upholding the identity of the pseudo and true diphtheria bacilli, and those of Escherich, maintaining their diversity.

Roux and Yersin found in a hospital for children in Paris, where cases of diphtheria occurred from time to time, that 15 out of 45 children contained in their healthy throats non-virulent bacilli resembling the Loeffler bacillus. In a village in France, by the coast, where no diphtheria had been present for a long time, they made cultures from the healthy throats of 59 children living in a school. In 26 of these non-virulent bacilli were found.

In an examination of the throats of the attendants in a diphtheria hospital, non-virulent bacilli were found once. Thus in 114 healthy throats the non-virulent bacilli were found 42 times. In all of these throats the bacilli were present in very small numbers. They found these same bacilli twice in six children with mild sore throats, and five times in seven children sick with measles. It should be noted that these examinations were made chiefly in a children's hospital and in a children's school, in both of which the children were confined together for considerable periods of time, and the direct transmission of these bacilli from one throat to another would be likely to occur. The unusually large percentage of children in which they were found might thus be accounted for.

The bacilli found, when studied in pure culture, differed somewhat from each other. The majority were identical in all their characteristics with the Loeffler bacillus, except for their lack of virulence. The minority resembled those described by Hofmann, being shorter and thicker, and growing at a lower temperature than the characteristic forms. They made an important observation, showing that the non-virulent bacilli, when grown in bouillon, caused the same changes in the reaction as the virulent forms—namely, from alkaline to acid in 48 to 72 hours, and later, back again to alkaline in the course of some weeks. These changes were

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found to occur even more rapidly in the cultures of the non-virulen bacilli. They regarded the occasional slight differences in growth, shape and staining as too slight and inconstant to separate the virulent fron the non-virulent bacilli. Animal experiments alone sufficed to determin the question of virulence, and they regarded as arbitrary a division founde upon the reaction to inoculation of the guinea pig; since they found tha bacilli from cases of diphtheria may possess every degree of virulence from those which cause death within twenty-four hours to those whic cause only a temporary œdema. With these variations it is a difficul matter to determine what should be the proper line of division betwee the virulent and the non-virulent bacilli.

To fully prove that these bacilli belong to the same species, they believe it is necessary to derive non-virulent bacilli from the virulent ones, and to give virulence to those entirely lacking it.

They found that it was possible to produce an attenuation of the virulence of the bacilli in a number of ways. For instance, if a current of sterile air is kept passing through a broth culture of diphtheria bacilli, maintained at a temperature of 39½° C., after about two weeks some of the bacilli begin to lose their virulence, and at the end of about four weeks all of the bacilli have lost all of their virulence and produce non-virulent cultures. A little later the bacilli remaining in the culture die.

They also found that if from time to time cultures were made from dried bits of membrane, a period finally came when the bacilli, although alive, had become non-virulent. Thus they had fulfilled the first condition.

The attempt to restore to bacilli the virulence which they had entirely lost was not so successful. They found it possible to greatly increase the virulence of bacilli by injecting them together with a virulent culture of the streptococcus of erysipelas. The bacilli obtained from animals which had succumbed to this double inoculation were found to have fully regained their virulence. They were unable, on the other hand, to give virulence to those bacilli which had been artificially attenuated so completely that they possessed no virulence whatever, or to those which had none originally when obtained from the throat. Thus, of the two proofs necessary to establish the identity of the virulent and non-virulent forms, they had obtained the first fully, the second only partially.

As additional proof of the indentity of the virulent and non-virulent bacilli, they brought forward the fact that they found the latter more frequently in patients recently convalescent from true diphtheria than in those who had never had the disease, and that the bacilli which had artificially been deprived of their virulence frequently were changed in other respects, so as to resemble in all ways the bacilli which were originally lacking in virulence. From their studies they concluded that the non-virulent and virulent bacilli were one and the *same* form of bacteria.

If we now turn to the work of Escherich, we find an able presentation of the view that the virulent and non-virulent bacilli are *different* varieties of bacteria.

He first lays stress on the methods to be employed in testing the virulence. He advises that the animal inoculations be made always from bouillon cultures, which have been grown for forty-eight hours at 37½° C., and that the amount of the culture be regulated by the size

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of the animal. With these precautions he found the bacilli from every case of diphtheria examined to be fully virulent, and in a few cases, where he obtained characteristic bacilli from the healthy throats of persons exposed to diphtheria, he found them also to be virulent.

Escherich did indeed find in a moderate number of throats of persons not suffering from diphtheria a bacillus similar to those described by Hofmann. Thus in Munich he found this non-virulent bacillus in two throats out of 70, and in Graz in 11 out of 250, or 13 times in 320 cases. These bacilli, however, all possessed certain cultural and morphological characteristics which were sufficient to separate them from the virulent bacilli. They were, as in some described by Hofmann, plumper and shorter than the Loeffler bacilli, and more uniform in size. The growth on agar was more luxuriant and whiter than is the case with the diphtheria bacilli. He noticed two new points of difference which seemed to him important. The pseudo-diphtheria bacilli, when spread on a cover glass, lie in parallel rows, while the diphtheria bacilli lie at every angle and in the most varied positions. The second difference was still more marked. He found, as had all others who had noticed this point, that the bacilli in their growth in alkaline bouillon changed the reaction of the bouillon to acid in the course of forty-eight hours. The amount of acid formed differed in different cultures, and had no relation to the degree of virulence. He then noticed that the pseudo-diphtheria bacilli always made the bouillon more alkaline instead of acid. Therefore, if at the end of forty-eight hours litmus was added to the different bouillon cultures it turned red in the virulent ones, and blue in the pseudo-diphtheritic non-virulent ones. Although this will be referred to again, it should be noticed that this difference in reaction was not found by Roux and Yersin in the cultures of non-virulent bacilli tested by them.

Escherich, in conclusion, states his position as follows :—"Since we "have found constant cultural differences between the true and the "pseudo-diphtheria bacilli we can give the pseudo-diphtheria bacilli no "diagnostic value. We do not find it to be a frequent inhabitant of the "mouth. Chronic throat inflammations and measles seem to render the "throat more liable to its invasion."

He says, in referring to the views of Roux and those who agree with him, that if we believed in the common transformation of a non-virulent bacillus, frequently inhabiting the mouth, into a virulent bacillus, our hopes of combating the spread of the contagion of diphtheria would be greatly diminished. We should then be compelled to attempt to isolate many healthy persons, and we should find it far more difficult to render a diagnosis in diphtheria by means of bacterial cultures. He did not find, as Roux and Yersin and Fraenkel had, that it was possible to determine from the abundance of the colonies of bacilli present whether they were composed of virulent or non-virulent bacilli.

If we review the literature of this subject we find that some investigators have adopted views similar to those of Roux and Yersin, others to those of Escherich, and still others have been forced to content themselves with the position of Hofmann, viz., that we are not yet in a position to affirm whether all these bacilli are of one or of different species of bacteria.

Up to the present time the results, so far as they are known to the writers, are given in the following table :—

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	Morphology.	Growth in Bouillon and Reaction.	Growth on Blood Serum.	Growth on Agar.	Frequency met with.	Opinion as to the Nature of the Bacilli.
VON HOFMANN..	Some bacilli identical with those of Loeffler, others were shorter, thicker, and more uniform in size	Similar to virulent.....	Sometimes identical with Loeffler bacilli; again found in larger and somewhat whiter colonies	Grows more luxuriantly, and spreads more on the surface. May become of a dirty brown colour in central part of colonies	In 45 throats, comprising some healthy and some the seat of non-diphtheritic inflammations, they were found in 26	Is doubtful whether these non-virulent bacilli belong to the same species as the virulent diphtheria bacilli, or whether they are of a different species
LOEFFLER	Somewhat larger than virulent bacilli, and more tendency to produce swollen ends	Similar to virulent.....	Similar to virulent.....	Colonies had less jagged edges and were of a whiter hue	Once with virulent bacilli from a case of diphtheria	Believes them to be of a different species, but only to be separated by animal cultures
ESCHERICH	Bacilli shorter, plumper and more uniform in size. When a drop of bouillon culture is smeared on a cover glass the bacilli are found to lie in parallel rows	More luxuriant growths with tendency to cause cloudiness. When grown in neutral litmus bouillon the litmus turns blue after two or three days	Fairly characteristic, but apt to be a more luxuriant and whiter growth	Grows more luxuriantly, and spreads more on the surface. May become brownish in colour after some days	In Munich in 2 out of 70 In Graz in 11 out of 250 healthy throats, and those the seat of non-diphtheritic lesions examined } 13 in 320	Believes that they have no relation to the virulent diphtheria bacilli, and that they can be separated pretty accurately by cultural differences
BECK	Shorter, plumper bacilli, as a rule, but some more like virulent bacilli	Quicker and more luxuriant in growth	Somewhat more luxuriant, and of a more yellow colour	Colonies less jagged on margin, and more yellow in hue	In 36 well children found in 22; in 41 non-diphtheritic affections in 14, or in a total of 107 found them in 36. Also along with virulent bacilli in true diphtheria	Believed that the non-virulent forms found by him were of a different species from the virulent, and were saprophytic in nature
KOPLIK— 1st paper	Short, plump, uniform in size. Take a more uniform stain	More luxuriant, cloudy at first, afterward clearing, with abundant deposit; bouillon	More luxuriant and spreading	More luxuriant and spreading	In four mild throat inflammations	Are of a different species from Loeffler bacilli.

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KOPLIK—2nd paper	Identical in form and size, with virulent and characteristic stain	Cloudy; less abundant growth in bouillon to which glucose has been added; bouillon alkaline after 48 hours	More luxuriant, opaque, and whiter growth	More luxuriant, opaque, and whiter growth	In two following true attack of diphtheria. For first three weeks found virulent bacilli, then for two weeks there were non-virulent forms	—
ROUX & YERSIN	Majority identical with virulent bacilli. The majority of shorter, plumper, and more uniform variety	Characteristic, except for slight cloudiness. Changes of broth were same as in virulent forms, but somewhat more rapid	Same as in virulent ...	Same as in virulent, varying within the limits noticed in different virulent cultures	In 104 healthy children's throats found 41 times. Ten adults once. In six mild throat inflammations twice; in seven sick with measles five times	Believed the non-virulent to be of the same species as the virulent; they were simply an attenuated form.
ABBOTT	Bacilli from three cases were identical with virulent forms; from one they were larger than the virulent average	Growth in bouillon same as in virulent forms, except the changes from alkaline to acid, and later back again to alkaline were more rapid than in case of virulent bacilli	Characteristic	Two of four characteristic, one more luxuriant in growth, and one giving colonies with darker central portion	Four times in 53 throats. Some healthy, others the seat of moderately severe inflammations	In doubt. *Note.*—The bacillus growing more luxuriantly on agar and in layer bacilli gave a dirty brown layer on potato.
FRAENKEL.....	Identical with virulent forms	Characteristic	Characteristic	Characteristic	In number of healthy conjunctivæ and in some cases of mild tonsillitis and with virulent bacilli in diphtheria. Figures not given	Believes the virulent and non-virulent to be of same species, and includes under non-virulent some causing local reaction.
MARTIN	Short, plump bacilli...	—	—	More moist, luxuriant, and whiter. Grow at room temperature	In quite a number of mild diphtheria cases running in mild course	An attenuated form of the virulent diphtheria bacillus.

If we inspect closely the descriptions of the non-virulent bacilli, we find that there seem to be two which stand out distinctly as separate varieties with which the others can be grouped.

1st. Bacilli which are in all respects, except as to virulence, identical with the Loeffler bacillus, and which like it produce an *acid* reaction in their growth in broth cultures.

2nd. Bacilli which are shorter, plumper, and more uniform in size than the Loeffler bacilli, and which produce an *alkaline* reaction in broth cultures.

As we look over the tables we see that some observers have chanced to find one of these varieties, some the other, and some both. This has led to the present confusion.

In order to study these various bacilli, and to clear up, if possible, some of the questions connected with their classification, cultures were made upon blood serum from 330 healthy throats.

When any of the varieties of bacilli described above were discovered in the cultures, they were plated out, obtained in pure culture, and, in the great majority of cases, tested as to their virulence on guinea pigs. The results of these studies are given in the tables below. The bacilli formed may be divided into three groups.

1. Bacilli characteristic in growth, producing acid in bouillon, but having no virulence.

2. Bacilli not characteristic in growth, producing alkali in bouillon, and having no virulence.

3. Bacilli characteristic in growth, producing acid in bouillon, and having virulence.

Healthy Throats where there has been no History obtained of direct contact with Diphtheria.

From where.	Total Cases. Nos.	Virulent Characteristic Diphtheria Bacilli.	Non-Virulent Characteristic Diphtheria Bacilli.	Non-Virulent Pseudo-Diphtheria Bacilli.	Negative.	Not fully Worked out.
N.Y. Dispensary (N.Y.)	1 to 151	3	12	21	115	25
Northern Dispensary	152 to 163	—	—	—	12	—
Vanderbilt Clinic (V.C.)	164 to 189	0	2	2	22	3
Throughout the city	190 to 193	0	4	0	0	0
College Phys. & S. (P. & S.)	194 to 242	0	2	3	44	4
N.Y.F.H. Dispensary (F.S.)	243 to 257	0	0	1	14	1
Orthopædic Hospital (O.H.)—						
Female Ward...	258 to 267					
Male Ward.....	268 to 275	0	3	0	15	2
N.Y. Foundling Hospital (N.Y.F.)	276 to 330	5	1	0	49	1
Totals	330	8	24	27	271	36

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A Comparative Table of

	Non-Virulent Diphtheria Bacilli.		Pseudo-Diphtheria Bacilli.	
Case No. and Source.	N.Y. 66 Throat.	N.Y. 72 Throat.	P. & S. 30 Throat.	N.Y. 101 Throat.
Examination of primary culture	Abundant large characteristic diphtheria bacilli	Abundant characteristic diphtheria bacilli	Typical pseudo-diphtheria bacilli	Abundant short even-stained bacilli (pseudo-diphtheria)
Growth in pure culture on serum at 37½ C.	Characteristic...	Characteristic...	Fairly characteristic. Short even-stained bacilli	Characteristic. Short even-stained bacilli
Agar	Fairly typical colonies	Not typical colonies	Coarsely granular [illegible] rough borders, and of about equal thickness throughout; brownish hue by transmitted light	Fairly typical. More [illegible]ily pigmented and uniformly thick than is characteristic of the virulent bacilli. Colonies nearly circular with even borders
Growth in alkaline glucose bouillon	Characteristic acid at end of 22 hours	Not characteristic. Broth cloudy for two days, acid end of 48 hours	Typical growth in rather coarse grains. Alkaline reaction end 48 hours	Formation of thin pellicle and slight diffuse cloudiness. Alkaline reaction at end 48 hours
Virulence in guinea pigs	G. Pig 216 gms. 1·33 c.c. No reaction	G. Pig 164 gms. 1·33 c.c. No reaction	G. Pig 405 gms. 3 c.c. No reaction	G. Pig 400 gms. 2 c.c. No reaction
Clinical notes...	Bronchitis. Diph. in house three weeks previously	Int. catarrh. No history of contagion	Healthy throat	Bronchitis. No contagion
Sex	F	F	—	M
Age	One year.....	15 months	27 years	10 years

In the above table we find that bacilli possessing all the characteristics of the virulent diphtheria bacilli, except that of virulence, were found in 24 cases, namely :—

Nos. 7, 33, 52, 63, 66, 72, 103, 105, 110, 114, 124, 132, 188, 189, 190 191, 192, 193, 198, 212, 258, 259, 260, 297.

These bacilli were abundant in the primary cultures from 17 cases and present in small numbers only in the cultures from 7.

They averaged a little longer than the virulent bacilli from the cases of suspected diphtheria examined on the same days. In broth the bacilli from 13 of the 24 cases grew characteristically, while from 6 they caused a more or less dense cloudiness. It was found, however, that exceptionally the virulent bacilli produced the same effect, though never to the same degree, as the bacilli from case 191. In 5 cases the bacilli were not grown in broth. In all the cases in which broth cultures were made (19), the bacilli caused acid reaction in their growth. When their acid-producing power was compared with that of an equal number of virulent cultures, no marked difference could be noted. Some virulent

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bacilli were found to produce more acid than the non-virulent ones, while others produced less.

Upon blood serum the bacilli grew in a manner characteristic of the Loeffler bacillus.

Upon agar, the bacilli from eleven cases grew in the characteristic manner, while from seven they grew in a less typical manner, but always in ways seen exceptionally in the virulent form. Guinea pigs were inoculated with the bacilli from fifteen cases.

For this purpose half-grown guinea pigs were employed, and they were inoculated under the skin with one-half per cent. of their weight of a forty-eight hour broth culture. In only one animal was there any appreciable reaction, and in this the local induration caused passed away within four days. A very slight degree of immunity was given to some of the pigs by the injection.

Two hundred and eighty of the 330 cases from which cultures were made were children under twelve, while 50 were adults. In only nine of the *twenty-five* were there present noticeable pathological changes in the throat, such as enlarged tonsils. The bacilli persisted in four of the throats examined for four weeks, in one for three weeks, in three for two weeks, and in some of the others for shorter periods.

In 21 cases bacilli were found corresponding to those described by Hofmann and Escherich, and pictured by Koplik in his first article. These were smaller, shorter, thicker and more uniform in size than the Loeffler bacilli, and always formed alkali in their growth in bouillon. These bacilli were never virulent in animals. Guinea pigs were inoculated with large amounts of broth culture of bacilli obtained from eight cases without showing any reaction.

As was shown in the table, virulent characteristic diphtheria bacilli were found in eight of the 330 cases.

They were in all probability derived from mild cases of unrecognized diphtheria, or from healthy children who were carrying the bacilli in their throats. The number of such infected children is indicated by the results of studies described in the following pages.

The Presence of Virulent Diphtheria Bacilli in Healthy Throats of Persons who have been brought in contact with Diphtheria.

The search for the origin of obscure cases of diphtheria has revealed the fact that it is possible for the human throat to become the habitat of the virulent Loeffler bacillus without any visible lesions resulting. Thus Loeffler found the virulent bacillus once, and Fraenkel twice; Escherich found it in several cases, in one of which the history is so significant as to be worth repeating. It was noticed that among the children coming under the care of a certain nurse a number of cases of diphtheria were developing. A bacteriological examination being made, her throat was found to contain very numerous virulent diphtheria bacilli. These remained present and virulent for weeks. A similar and interesting case is reported by Feer. In a diphtheria epidemic occurring in a hospital ward, due to a single infection, the throats of seven children became infected. The infection caused fatal diphtheria in four, an acute angina

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without membrane in two, and no symptoms whatever in one. In all of these the bacilli were abundant and equally virulent. Many similar examples have been met with by myself (Park).

A very interesting investigation has been carried on to determine how frequently the throats of healthy children become infected in families where one is sick with diphtheria, and where little or no isolation is possible.

As will be seen by the following tables the throats of the members of fourteen families, in which there were forty-eight children, were examined. In fifty per cent. of these diphtheria bacilli were found, forty per cent. developed later, to a greater or less extent, the lesions of diphtheria. In considering the high percentage of cases in which this virulent Loeffler bacillus was found, it must be remembered that in these families the conditions were the best possible for the transmission of the contagium.

In numerous instances cultures have been made from the healthy throats of children in families where the diphtheria case was well isolated ; in such cases the bacilli have been found in less than ten per cent. of the children.

Family.	No. of Cases examined aside from the original case of Diphtheria.	L. Bacilli found in	L. Bacilli not found in	Remarks.
A.	1	0	1	Isolation
B.	3	3	0	No isolation : all three cases subsequently developed diphtheria
C.	2	1	1	
D.	1	1	0	No isolation
E.	3	1	2	
F.	4	1	3	Isolation partial
G.	5	3	2	Isolation partial
H.	4	3	1	No isolation
I.	4	1	3	
J.	8	3	5	Isolation partial
K.	4	1	3	Isolation partial
L.	3	1	1	Isolation partial
M.	5	3	2	Isolation fair (virulent)
(2) N.	1	1	0	(Virulent)
14.	48	24	24	

Of the above cultures, in which the Loeffler bacilli were found in six, the virulence was tested in the usual way. The results are stated in the following table :—

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Family No.	Case No.	Amount Bouillon cult. inoc.	Guinea Pig lot.	Virulence.	Clinical history.
B.	1	1·33 c.c.	337 gm.	Died in C. 40 hrs.	Developed fatal diph. one day after cult. was taken
G.	2	1 c.c.	205 gm.	Died in 44 hrs.	Developed set. tonsillar diph. two days after cult. was taken
H.	3	1·33	202 gm.	Died in 48 hrs.	No subsequent development of diphtheria
K.	4	1·33	300 gm.	Died in C. hrs.	No subsequent development of diphtheria
M.	5	1·66	490 gm.	Died in C. 40 hrs.	No subsequent development of diphtheria
N.	6	1 c.c.	250 gm.	Died in C. 40 hrs.	No subsequent development of diphtheria

It may be interesting to detail here two instances out of many observed in which the virulent bacilli of diphtheria derived from healthy throats have been the cause of diphtheria in others.

1. A child was admitted into a hospital ward in an anæmic condition, and with a chronic coryza. Five days later four children in his neighbourhood developed diphtheria. Two of these died. In seeking the cause of the diphtheria suspicion was directed to the child by a slight nasal discharge. Bacteriological examination showed that this secretion contained many diphtheria bacilli. On further examination it was found that the child came from a family in which three weeks before there had been a case of diphtheria.

2. In a family of eight children one child sickened with diphtheria; a baby was sent to a neighbour. The next day cultures showed that this baby, as well as two others, were infected with diphtheria bacilli. The three healthy infected children, as well as the sick one, were at once quarantined, but already one of the family to which the baby had been sent had contracted diphtheria from it.

The practical value of bacteriological examinations of the throats of healthy children in families where isolation has not been carried out in the first days is further shown by the fact that those children in whom the bacilli are found are extremely apt to develop diphtheria in the course of a few days, when no cleansing treatment is adopted, while they seem much less liable to do so if kept under treatment.

The Detection of the Virulent Bacilli in Throats prevents the Dissemination of Diphtheria by allowing us to isolate those infected.

A very striking instance of this was the following: In a family of four children one was sick with diphtheria. The Department inspector found three other children in the same bed with the sick one, who was constantly spitting upon and soiling the bedclothes. He made cultures from these three children, whose throats appeared healthy, as well as from the sick one; all contained abundant characteristic Loeffler bacilli. (Those were later

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shown to be virulent by inoculation of guinea pigs.) When the inspector visited the same family three days later, he found two of the previously healthy children had meanwhile sickened and died, and that the third was severely ill. This child finally recovered.

From the observations detailed above we cannot escape the conclusion that *all members of an infected household should be regarded as under suspicion*, and in those cases where isolation is not enforced, the healthy as well as the sick should be prevented from mingling with others until cultures from the throat have shown the absence of bacilli or a sufficient lapse of time gives the presumption that they are not carriers of the contagium.

Summary and Conclusions.

We have found that children, and to a less extent adults, who are frequently brought in direct contact with true cases of diphtheria very often receive the diphtheria bacilli into their throats, and that these bacilli may persist and develop in these throats for days or weeks. In some cases we have found that true diphtheria followed the appearance of the bacilli in the respiratory passages, while in others no disease developed, though they might be the source of diphtheria in others.

The examination of 330 healthy throats in which no contact with diphtheria was known revealed the presence of virulent bacilli in but eight cases, two of which later developed diphtheria.

We must conclude, then, that virulent diphtheria bacilli are to be found in a small proportion of healthy throats throughout the city, and that they have been acquired either directly from diphtheria cases or from those who have been in contact with them. The examinations of the 330 healthy throats showed that in 24 bacilli existed in every way identical with the Loeffler bacillus, except that they were not virulent in animals. As the bacilli in true diphtheria cases are known to gradually lose their virulence, and as this loss of virulence can be caused artificially, it seems to the writers that they should be regarded as true diphtheria bacilli, which have lost their virulence.

The examination of the same throats showed that in 27 there were bacilli present, which, although closely resembling the diphtheria bacilli, showed constant differences not only in size and manner of staining, but in the chemical products of their growth. These bacilli should be differentiated from the diphtheria bacilli, and are properly designated as pseudo-diphtheria bacilli.

The results of these investigations, together with those recorded by others, seem to indicate that there are at least two varieties of bacteria, and perhaps more, to which the name pseudo-diphtheria bacillus has been given. It seems to us that these different bacilli should be separated in name as they are in fact.

First: There are bacilli which differ in no respect from characteristic diphtheria bacilli, except that they lack virulence. As Roux and Yersin, Fraenkel and others have pointed out, this loss of virulence occurs naturally at times (virulent and non-virulent bacilli even existing at the same time in a throat), and can also be produced artificially.

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It seems to us that these bacilli should be classed with the virulent diphtheria bacillus, in spite of the fact that their lack of virulence gives them an entirely different relation to the disease diphtheria. The twenty-four cases in which these bacilli were found never developed diphtheria while they remained under observation, nor was any case of diphtheria ever traced to them. This seems to accord with the experience of others.

With the diphtheria bacilli should be classed temporarily such exceptional bacilli as those found in the two special cases described above, for we know that even among virulent bacilli there are marked differences in the luxuriance of their growth.

Second: There are the bacilli especially described by Escherich and photographed by Koplik. These are so uniform in their peculiarities in staining, size, shape and the production of an alkali instead of an acid, that there seems to us to be even more reason to separate them from the diphtheria bacillus than there is, for example, to separate the colon bacillus from that of typhoid.

We have never found bacilli possessing these peculiarities to be virulent, nor have they seemed to have any connection with diphtheria. It seems to us that to these bacilli alone the name pseudo-diphtheria bacillus should be given.

The few bacilli which do not seem to come under either of these divisions must await further study before being classified.

Some of the Methods by which Diphtheria is Transmitted.

The facts brought out by the investigations of the Department throw important light on the manner in which diphtheria is transmitted.

As related to this question, let us consider first very briefly what is known of the duration of life of the Loeffler bacillus outside of the body.

In actual experiment the Loeffler bacillus has been found to live for long periods of time—namely, by Hofmann on blood serum for 155 days, by Loeffler[24] and by us for 7 months, and in gelatine by Klein[25] for 18 months. The bacilli have been found to live in bits of dried membrane by Loeffler for 14 weeks, by us for 17 weeks, and by Roux and Yersin for 20 weeks. Dried on silk threads, Abel[26] reports that they may sometimes live for 172 days, and upon a child's plaything, which had been kept in a dark place, for 5 months.

As examples of the manner in which diphtheria may be contracted, he gives the following from Johannessen:—

A teacher developed diphtheria from passing the night in a room in which three weeks before a fatal case had occurred. A child developed diphtheria after putting on the clothing worn by a child which had died of diphtheria two months before.

In a number of isolated dwellings diphtheria developed nearly a year after previous outbreaks without there being any apparent possibility of a new infection taking place from outside. We have met with a number of cases where the infected bedding or clothing has undoubtedly been the source of the contagion.

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Sources from which Virulent Bacilli may be received.

1. From the pseudo-membranes, exudate or discharges from diphtheria patients.

2. From convalescent cases of diphtheria in whom the virulent bacilli persist in the throat.

3. From the healthy throats of individuals who have been in contact with others having virulent germs on their person or clothing. In these cases the bacilli have lived and developed for days or weeks in the healthy throats without causing any lesion.

When we consider that it is only the severe types of diphtheria that remain isolated during their actual illness the wonder is, not that so many but that so few persons contract the disease. This seems to be more remarkable when we observe that in a city like New York the whole tenement house district at least is an infected area. This has become evident from the observations made by the Department.

It has been the practice of the Department during the last year to plat upon a city map the location and date of every case of diphtheria in which the diagnosis had been settled by bacteriological examination. After several months the first map presented a very striking appearance. Wherever the densely crowded tenements were located, there the marks were very numerous, while in the districts occupied by private residences very few cases occurred. It was also apparent that the cases were far less abundant, as a rule, where the tenements were in small groups, than in the regions of the city where they covered large sections. At the end of six months there were square miles in which nearly every block occupied by tenement houses contained marks indicating the occurrence of one or more cases of diphtheria ; and in some blocks many cases (15 to 25) had occurred.

As the platting went on from time to time the map showed the infection of a new area of the city, and thus often a local epidemic would be started. It was interesting to note two varieties of these local epidemics ; in one the subsequent cases evidently were from neighbourhood infection, while in the second variety the infection was as evidently derived from schools, since a whole school district would suddenly become the seat of scattered cases. At times, in a certain area of the city, from which several schools drew their scholars, all the cases of diphtheria would occur, as investigation showed, in families whose children attended one school, the children of the other schools being for the time exempt.

Another fact noted, perhaps as important as the foregoing, was that with the most careful inquiry it was impossible to find, in about one half of the cases, any connection with preceding cases of diphtheria.

The two following histories are instructive as showing that special conditions which are largely unknown to us determine the occurrence or escape from diphtheria under exposure. Two children in a family were taken sick with diphtheria and removed to the hospital. The servant went to another family, where the youngest child developed diphtheria a week later. In the meantime a case developed in the family living in the next apartment. There were in this family three other children

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which were not isolated at all from the sick child. None of these developed diphtheria.

The child of a man who kept a candy store developed diphtheria; there were four other children in the family and these were in no way isolated from the sick one, still none of them developed diphtheria, but children who bought candy at the store, and other children coming in contact with the latter in school, developed diphtheria. The secondary cases ceased to develop so soon as the candy store had been closed.

Many similar histories could be given to illustrate the fact that the majority of persons, and even the majority of children, are not very susceptible to diphtheria, and that in addition to receiving the germs of the disease into the respiratory passages they must be in a condition favourable to the development of the disease.

It seems to be generally true that the more malignant a case of diphtheria is, the more likely it is to cause diphtheria in others. This may be due to the high grade of virulence possessed by the bacilli, or to the peculiar association of other micro-organisms in the membrane, or to the wider dissemination of the infectious matter through the discharges.

It is also well known that young children are much more susceptible to diphtheria than older persons. It is comparatively rare for the parents of children sick with diphtheria to contract the disease, although in nearly every case they must at some time receive the germs into their throats.

Should Cases of False or Pseudo-Diphtheria be Isolated and kept under the Supervision of the Health Department?

In the general circular issued by the Department the position was taken that cases which prove to be false diphtheria will not be kept under the supervision of the Department. Some, who approve heartily of the rest of the work of the Board in its dealings with diphtheria, believe that in this step it has proceeded too far, and that the pseudo-diphtheria cases, though less contagious than the true, are yet sufficiently so to render isolation and supervision necessary. From the experience obtained in the diphtheria hospital, it was believed that these cases were so little, if at all, contagious, that visiting by Department inspectors was unnecessary. Nevertheless, to make assurance doubly sure, 450 cases of false diphtheria, as nearly consecutive as possible, were investigated, all sources of contagion sought for, and the cases followed up for two weeks after complete convalescence. In none of these was isolation or after disinfection enforced by the Health Department. This is such an important question, that the results of the investigation of 100 consecutive cases are given below in tabular form. As a comparison, a similar table will be given of fifty consecutive cases of true diphtheria which were taken from the same district, and at the same time of the year as the first fifty of pseudo-diphtheria cases.

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Summary of Tabulated Cases.

	Table 1. Pseudo-Diphtheria.	Table 2. Pseudo-Diphtheria.	Table 3. True Diphtheria.
History of contact with other cases ...	7	7	33
No history of contact	49	50	27
Families in which more than one case developed	5	4 [1]	13
Recovered	56	53	46
Died	0	4 [2]	17
Cases complicated by scarlet fever	4	6 [3]	—

We find, therefore, in 113 cases of false or pseudo-diphtheria occurring in 100 families that 14 occurred at the same time with or shortly after some other case, and that it is possible to assume that the disease had been directly communicated to them. In 9 of the 100 families more than one case developed. In these, as in the other 350 cases of pseudo-diphtheria investigated, it did not seem that secondary cases were any less liable to occur where the primary case was isolated than when it was not. In this connection we must remember that mild throat inflammations are very frequent in the early spring months, and that it is quite possible where two cases occurred in a family together or within a short period of each other that they may have both been due to some common exposure rather than to direct transmission. The presence in all healthy throats of streptococci renders this assumption almost a probability. The presence of the same germs in both healthy throats and those suffering from pseudo-diphtheria prevents us from deciding the point by bacteriological examinations.

A good illustration of the difficulty in determining whether these cases are communicable is the following :—

In a family of eight, there were a mother, aged 45, six children, whose ages ranged from 25 to 10, and a grandchild aged 2. These all lived on the top floor of a tenement. Two days before being visited by the inspector of diphtheria a heavy wet snow had fallen, which, as the roof leaked, caused the walls to become very damp. The next morning four of the children were attacked by more or less severe tonsillitis, which later developed follicular deposits or croupous patches, and on the following day the baby had an attack of croup. All recovered, and no further cases developed in the tenement. Here the exposure to dampness seems certainly to be the explanation of the first four cases, but the last—that of the little child with laryngitis—might with equal justice be considered as due to the dampness, or as communicated from the others.

Even if further investigation should seem to prove that the 122 cases of pseudo-diphtheria, which were found to have had some connection with other mild sore throats, were due to contagion, and not to the simultaneous

[1] Two had scarlet fever.
[2] Three of which had scarlet fever.
[3] Six others had been in contact with scarlet fever.

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effects of atmospheric or other deleterious conditions, there would still be an important practical objection to enforced isolation. Every one of these 15 cases, except the three having scarlet fever, was mild, and, indeed, leaving out of consideration the cases which occurred as complications of scarlet fever, there was only one death in 103 cases of pseudo-diphtheria, and in this one, as has been said, there was no history of contact with other cases.

ABSTRACTS.

DIPHTHERIA, &c.

Chaillou and Martin. — *Clinical and Bacteriological Study of Diphtheria.* "Annales Institut Pasteur," Vol. VIII., No. 7.

AN interesting study based upon the bacteriological examination of 198 cases of suspected angina or laryngitis. In 99 cases of angina the authors found (1) 29 cases of non-diphtheritic angina without death, (5 cases of angina with coccus, 1 case of angina with pneumococcus, 4 with staphylococcus, and 11 with streptococcus). (2) True diphtheritic angina—benign : 30 cases, no death ; grave : 14 cases, 10 deaths. (3) Diphtheritic angina with association of other microbes : 26 cases, 18 deaths.

From 99 cases of laryngitis (croup)—(1) Non-diphtheritic without angina : 7 cases, 1 death. (2) Non-diphtheritic at the *début*, but becoming diphtheritic by contagion : 7 cases, 3 deaths. (3) Diphtheritic laryngitis : 85 cases, 55 deaths. *A. Cartaz.*

Bikeles.—*Anatomical Changes in Diphtheritic Paralysis.* "Lancet," Aug. 25, 1894, p. 450, from "Neurolog. Centralblatt."

NO changes were found in the peripheral nerves, but in the spinal cord, especially in the dorsal region, in the root-zone of the posterior columns, and also in the posterior part of the lateral columns, and in the anterior root fibres, degeneration was marked. The patient was forty years of age, and during convalescence four weeks after the attack he had nasal speech, difficulty in swallowing, paresis of the lower limbs, paræsthesia, ataxy, and loss of knee-jerk. *Dundas Grant.*

Moizard.—*Treatment of Diphtheria.* "Lancet," Aug. 18, 1894, p. 412.

THE local application of a five per cent. solution of corrosive sublimate in glycerine is made twice or thrice in twenty-four hours. In children below two years of age the strength is reduced to one in thirty or forty. Each application is preceded and followed by a clearance of the fauces by means of a dry tampon. Every four hours the throat is thoroughly douched with boric solution, lime water, or plain boiled water. He considers the "lesser frequency of manipulation" (? ABS.) an advantage presented by this over other methods. *Dundas Grant.*

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