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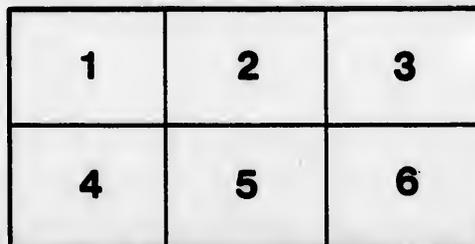
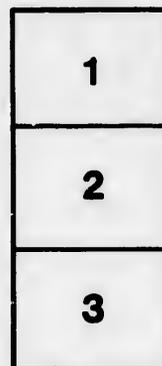
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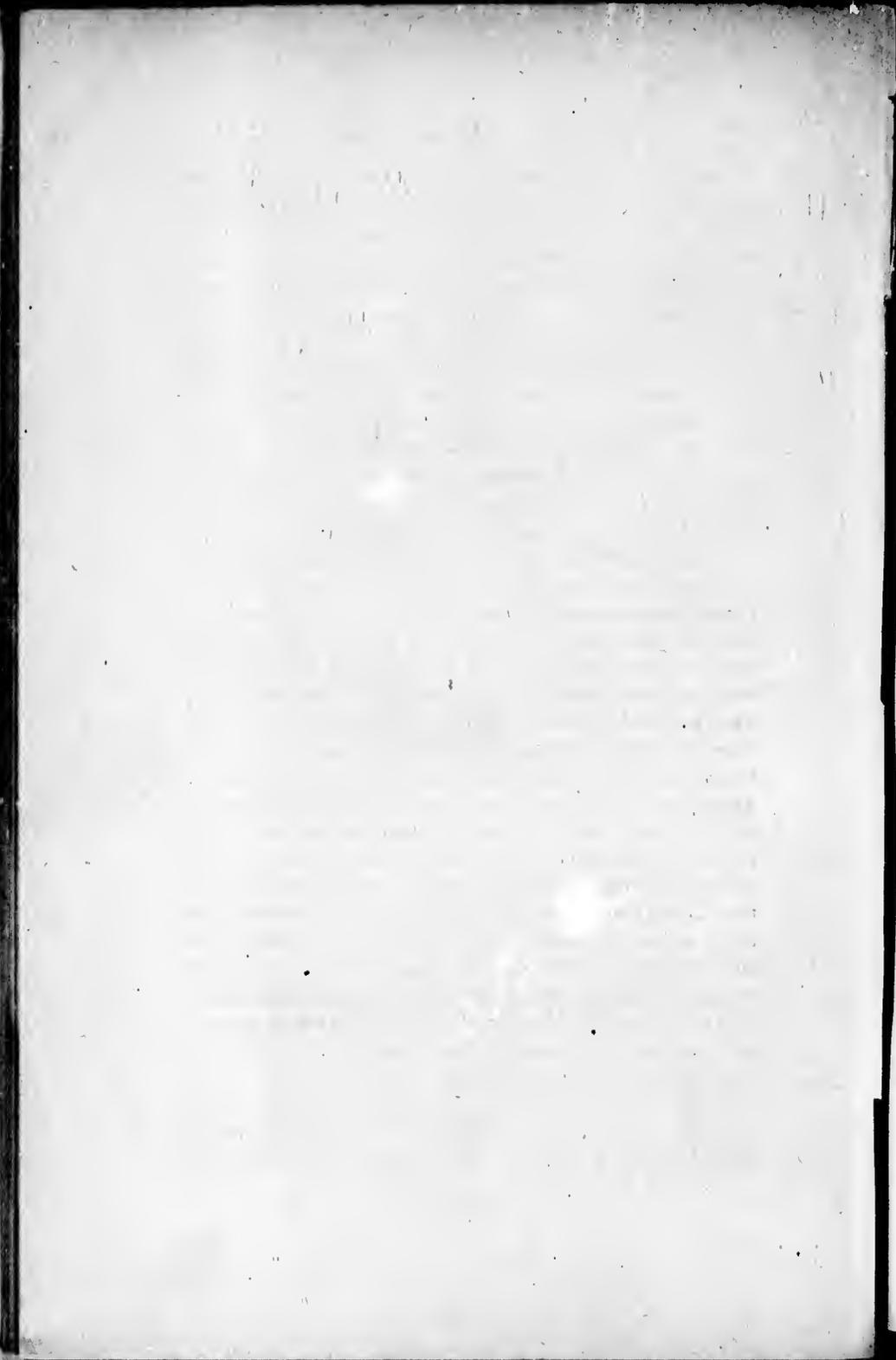
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NOTES ON THE BACTERIOLOGICAL STUDY OF DIPHTHERIA.*

BY WYATT JOHNSTON, M.D., MONTREAL.

My original object in studying cases of diphtheria bacteriologically was to settle the nature of a series of those doubtful cases in which a diagnosis is practically impossible by the ordinary means of observation, and where the occurrence of post-diphtherial paralysis or the outbreak of similar or more typical sore throats is the earliest positive proof of the true nature of the case. As I have found it impossible to continue the work on account of the difficulty in obtaining clinical histories of the cases, I wish to publish the results obtained from the examination of another series of cases, most of which were capable of being diagnosed as diphtheria without much difficulty.

Some time ago I showed before this Society cultures obtained from cases of diphtheria in which the growth had presented the typical characters of the Klebs-Loeffler bacilli. The main characteristics of this organism are :

(1) Rapid growth in serum at blood temperature leading to the formation of well characterized colonies in 16 to 24 hours.

(2) Peculiarities of structure, especially the presence of involution, bacillus forms having clubbed or swollen ends, with granular, unevenly stained protoplasm.

(3) Toxic effects, producing pseudo-membranous inflamma-

* Read before the Medico-Chirurgical Society of Montreal.

tions, followed by characteristic paresis, in cats and rabbits, and uniformly killing guinea-pigs in two to five days when injected subcutaneously, with production of necrosis, surrounded by local inflammations and œdema at the site of inoculation, and usually associated with more or less marked parenchymatous degenerations and areas of cell-necrosis of the viscera. This condition is distinguished from other forms of experimental septicæmia by the fact that bacteria are absent from the blood and viscera.

These toxic effects are most striking, and serve to distinguish this organism absolutely from all other species of bacteria. They depend on the fact that the diphtheria bacilli generate an albuminous poison or toxin which, when absorbed into the system, produces fever, cell necrosis and paralysis, which symptoms and lesions can be also brought about by the injection of sterilized cultures containing the *toxin* alone without any living bacilli. The primary local lesions in diphtheria are probably due both to the toxic action of the poisonous substances evolved and the presence of the bacilli.

The disease diphtheria is therefore complex in nature; the presence of the local exudation of false membrane and the toxic constitutional effects being each a necessary part of all cases of true diphtheria. As the diagnosis is naturally based upon the local inflammatory appearances, and as diphtheria is by far the commonest cause of pseudo-membranous inflammations, the term diphtheritic has come to be applied to all severe inflammations attended with the formation of false membrane and accompanied by necrosis, while the term croupous is applied to milder inflammations where there is no necrosis. It should be borne in mind that the terms diphtheritic and croupous are of anatomical and not of ætiological significance, as the neglect of this distinction has led to a great deal of confusion. Diphtheritic inflammation is most often caused by the disease diphtheria, but is not by any means invariably due to this cause, since every sloughing inflammation of a mucous surface presents diphtheritic characters and must be called, on anatomical grounds, diphtheritic. On the other hand, croupous inflammations have been shown by Paltauf-

Kolisko to be sometimes the effect of the action of the Klebs-Löffler bacilli, so that in this case we have a *croupous non-diphtheritic diphtheria*.

To prevent error, the terms diphtheria and diphtheritic should not be considered synonymous; and since the word diphtheritic has become too firmly established to be dropped from the vocabulary, it might be well if a suggestion of Dr. MacAllister (*Practitioner*, June, 1890) were adopted and the word *diphtherial* used always to express ætiologically in relation to the specific cause diphtheria, applying the term diphtheritic simply in its more general anatomical sense.

There is evidence to show that cases occur in which an anatomically genuine diphtheritic sore throat may be caused by other organisms than the Loeffler bacilli. Roux and Yersin (*Pasteur Annales*, July 1890) record with great care several such cases where the infection was due apparently to streptococci. Out of 80 cases of angina admitted to the diphtheria wards of the Childrens' Hospital, the bacilli were found in 61. Roux and Yersin had no scruples in classing the other 19 cases, in which no bacteria were found, as non-diphtherial angina. A much more remarkable series was that published by Dr. T. M. Prudden, where, in 24 cases of diphtheritic sore throat occurring in children, streptococci were found in 22 and staphylococcus aureus in 2, while the Loeffler bacillus was not met with in a single instance. It is important to note that these cases were obtained from an epidemic among children who were inmates of an institution in which scarletina and erysipelas were epidemic at the time.—(*Amer. Jour. Med. Sci.*, May 1889.)

A subsequent series of 12 cases, all fatal, by the same author (*N. Y. Medical Record*, April 18th, 1891), showed the presence of the virulent Loeffler bacilli in every instance, and a carefully prepared table appended, giving the total number of cases investigated in this manner by various authors up to date, shows that the Loeffler bacilli were found in 307 out of a total of 342 examined—about 90 per cent. The suggestion that the cause of diphtheria in America differed from that in Europe had been previously disproved by Prof. W. H. Welch and Dr. A. C.

Abbott, who, in a most exact study of eight cases of typical diphtheria occurring in Baltimore, found the Loeffler bacilli present in every instance.—(*Johns Hopkins Hosp. Bulletin*, Jan. 1890.)

That diphtheritic membrane can be produced amongst other causes by the streptococcus pyogenes is shown by the uniform presence of this organism in the diphtheritic endometritis occurring in puerperal fever. The only statements as to the absence of the Loeffler bacilli in diphtheritis of other regions than the throat is that furnished by Paltauf and Kolisko, who were unable to find the bacilli in this condition or in intestinal or cutaneous diphtheritis phagadana.—(*Wiener med. Wochenschrift*, No. 8, 1889.)

I have tabulated below the cases collected by Dr. Prudden and added my own cases. (I have not included in this table the cases where I was not able to obtain satisfactory material for examination, as I found in cases A 2 and A 3 that the bacilli might easily be missed if the secretions only were examined, and although found in portions of actual membrane).

OBSERVER.	REFERENCE.	No. of Cases Examined.	Loeffler Bacilli.	
			Present in.	Absent in.
Babes	<i>Zeitschrift f. Hygiene</i> , Bd. 5 . . .	42	42	0
D'Espine	<i>Revue Medicale de la Suisse, Romande</i> , 1888, No. 1	14	14	0
Ortman	<i>Berl. Klin. Wochen.</i> , 1889, No. 10	16	15	1
Spronek	<i>Central. f. Pathol.</i> , Bd. 1, p. 218.	7	7	0
Roux and Yersin	<i>Annales de L'Institut Pasteur</i> , Dec. 1888	15	15	0
Paltauf and Kolisko	<i>Wiener Klin. Woch.</i> , 1889, No. 6	50	50	0
Zarniko	<i>In Aug. Dissertation</i> , Kiel, 1889	20	18	2
Beck	<i>Zeitschrift f. Hygiene</i> , Bd. 8	52	50	2
Sorensen	<i>Nordiskt Medicinskt Archiv</i> , Bd. 18, No. 25	10	7	3
Escherich	<i>Cent. f. Bacteriologie</i> , Jan. 2, '90	22	20	2
Tangi	<i>Cent. f. Pathologie</i> , Hd. 1, p. 785.	18	18	0
Briegn and Fraenkel	<i>Berl. Klin. Wochen.</i> , Mar. 17, '90	22	22	0
Prudden (1st series)	<i>Am. Jour. Med. Scie.</i> , May 1889.	24	0	24
Welch and Abbott	<i>Johns Hopkins Hosp. Bul.</i> , vol. 2, No. 11	8	8	0
Prudden (2nd series)	<i>N. Y. Med. Record</i> , April 18, 1891	12	12	0
Johnston	<i>Montreal Med. Jour.</i> , Sept. 1891.	10	9	1
		342	307	35

The method of examining is very simple, and no one having an elementary training in bacteriology would have any trouble in carrying it out. Following the directions of Roux and Yersin

(*Annales Pasteur*, July 1890), I employed sterilized serum, obtained either from ox-blood or from hydrocele or pleuritic exudation. I can quite confirm the statements of these observers as to the advantage of this medium over agar-agar jelly, since by employing serum the colonies of the diphtheria bacilli are readily recognizable at the end of twenty hours, or even earlier if the serum be "improved" in the manner recommended by Loeffler, through the addition of one-fourth its bulk of a broth containing peptone, beef-tea and sugar. On the other hand, if agar-agar be employed, the colonies are never recognizable before the end of forty-eight hours, and show nothing strikingly characteristic before the fourth day. As the essential object of the examinations is to make an early and positive diagnosis, the saving of twenty-four hours would seem to be of vital importance in itself, but the serum method has also the advantage of permitting the diphtheria bacilli to bring their colonies to maturity before the other bacilli which are present have even commenced to form visible colonies. With agar, on the other hand, the two days needed for the appearance of the diphtheria colonies affords ample time for the development of the putrefactive forms, if these are present in any large number. The only advantage of the agar method is that the pyogenic staphylococci and streptococci which are usually present have more characteristic growth than on serum—a matter of secondary importance.

There is a current impression that the serum is difficult and troublesome to prepare, and this has led to its use being avoided in many laboratories when any other medium can be substituted. This idea is quite erroneous, as serum is as easily made as any of the other nutrient media—in fact, far easier than gelatine, if prepared according to the method given by Hueppe (*Centralb. f. Bact.*, July, 1887), which consists in coagulating and sterilizing the serum at once simultaneously. After the tubes are filled to a depth of one to two inches they are laid obliquely in rows in a thermostat, which is then heated till the inner temperature reaches 68° to 75°C. After half an hour or more at this temperature the tubes will be found to have coagulated, leaving the serum nearly transparent. The temperature can now be raised

Bacilli.

Absent
in.

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having
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Yersin

to about 90° by bringing the water in the jacket to the boil, and the tubes should be exposed to this heat for half an hour each day on three or four successive days, when, on placing them in the incubator in the usual manner, all but a few will be found to remain perfectly sterile. If Loeffler's serum is required, the serum is mixed with one-fourth its volume of Loeffler's bouillon before filling into the tubes. This addition does not interfere with the property of coagulating and remaining transparent. If blood serum is employed, care should be taken in collecting it that the clot is allowed to form before transporting the jars of blood. After standing forty-eight hours in a refrigerator or in a cool cellar, an abundant supply of clear serum can be obtained. The presence of small traces of hæmoglobin in the serum does not much impair its translucency, certainly not enough to render it unsuitable for the isolation of diphtheria bacilli.

The serum tubes could be prepared and kept in stock by druggists if the method ever comes into general use, which seems unlikely. The examination may be made from material taken direct from the throat by scraping the membrane, or, preferably, a piece of membrane may be detached by a pair of forceps or a swab of cotton wool. If the membrane has to be transported, it may be put into a clean, dry test-tube or folded up in blotting paper. To examine, it need only be moistened by a drop of sterilized water. A microscopical examination can be made by smearing the piece of membrane over the surface of a cover-glass, passing it three times through a flame. After drying and staining with a drop of any aniline stain, but preferably by Gram's method, the diphtheritic bacilli are seen as short thick rods (about the same length as tubercle bacilli), lying in little groups. These bacilli are present in enormous numbers in the early stages of diphtheria, but diminish rapidly in number as the membrane softens. The highly characteristic involution forms, which assume comma or club shapes with swollen ends, and present a protoplasm broken up into small granules, are only recognizable with a good immersion lens.

The cultures are made by drawing the infected needle in parallel lines along the surface of the serum. By treating two

or three tubes in this fashion, the infecting material at first abundant and producing a continuous dense growth, will in the second or third tube only implant a very few bacteria, so that the colonies can be studied separately. The tubes are to be kept at about body temperature for twenty-four hours, when minute white points appear on the surface of the serum, attaining about the size of pin-heads or of split peas. These, if found under the microscope to consist of bacilli, are probably the diphtheria bacilli, as the other forms would not have attained such size in so short a time.

The other colonies which attain such proportions in twenty-four hours are almost invariably found to be micrococci, usually the staphylococcus pyogenes.

I will not go further into the culture experiences with the bacillus than to say that I have been able, in all my cases, to confirm the statement first made by Welch and Abbott, of the Hygienic Institute, that the bacilli form an abundant invisible growth on potatoes, a medium stated by previous observers to be unsuitable for its cultivation. I also can substantiate the statement of Beck, that the agar cultures do not show the characteristic involution forms.

The method, however, has a serious defect, since an organism exists which is identical in size and appearance with the Loeffler bacillus, and grows on serum in a similar manner, though totally devoid of pathogenic properties. This is called the pseudo-diphtheria bacillus, and has been frequently found in the throats of healthy persons, as well as in follicular tonsillitis. The proof, therefore, is not absolute until substantiated by the inoculation of some susceptible animal. How far this pseudo-diphtheria bacillus would interfere with the method in practical work is not yet certain. Personally I have so far only met with the virulent or true bacillus.

The results of my examinations are as follows:—

Of nine cases examined in which the diagnosis of diphtheria could be made without much difficulty from the symptoms and the appearance of the throat, the Klebs-Loeffler bacillus was found in eight. In the case where it was not found, the con-

dition was one of an undoubted false membrane which contained, microscopically, large numbers of bacilli which appeared to be the organisms in question but did not appear in the cultures. When the specimen was taken the throat had just been freely sprayed with a solution of hydrogen peroxide, and the negative result may have been due to its disinfectant power of inhibiting the growth, though the fact that colonies of staphylococcus aureus appeared make that less likely. There was an anomalous course in this case, since the throat was found perfectly clear of membrane on the following day, preventing my repeating the experiment. In spite of this the disease appears to have been true diphtheria, as the nurse stated that the patient had a distinctly nasal voice when he left the hospital, ten days later. The bacillus was also found in an anomalous case where an extensive false membrane existed with almost no disturbance of the general health. In this case there was no paralysis.

In six cases where the diagnosis was doubtful, the bacilli were not found. Several cases of follicular tonsillitis and a case of scarletinal sore throat were examined with negative results.

I have divided the cases into two groups—(a) where the condition was clinically like diphtheria, and (b) anomalous cases. The cases are as follows, the first being given in detail and the remainder summarised in a table at the end of the article.

Case 1.—M. L., female, aged 21, admitted April 9th, 1891, into the female medical ward of the Montreal General Hospital, under Dr. Ross, with a suspicious-looking patch of membrane in both tonsils and a tiny membranous patch on the side of the uvula; temperature $102\frac{1}{2}^{\circ}$. Next day an extensive patch of dirty gray membrane was seen in the uvula and soft palate. Transferred to diphtheria ward. Seen on April 11th; temperature 100° ; had a patch of membrane on left anterior pillar of fauces. Discharged May 2nd; no paralysis.

Microscopic examination of the membrane showed an enormous number of short, thick bacilli, a few larger bacilli, and some clusters of micrococci.

Cultures in serum made on May 11th and kept at 35°C . showed on the following day numerous small, flat, white colonies

composed of short, thick bacilli, having the typical appearance of the Loeffler bacilli. Involution forms numerous. In agar plates small, flat colonies appeared on the second and third days, showing superficial thin concentric growth from a small central white spot. In stale culture in agar the growth, on first transplantation, was barely visible, but in subsequent generations the colonies became more distinct, forming always flat, circular, concentric, white growths on surface about the entry point of the needle. Microscopically the bacilli in the agar cultures were short, thick rods with rounded ends; their length was about 0.0015 to 0.002 mm. (one and a half to two-thousandths of a millimetre). None of the agar cultures showed involution forms.

April 28th, 1891.—Inoculated a very large, full-grown female guinea-pig with 0.7 ccm. of a watery suspension obtained from washing a seven days old culture in six per cent. glycerine agar-agar. Injected subcutaneously in right flank.

May 6th.—Found dead in cage; had been quiet and refused food for past three days. In right flank, at site of inoculation, induration and gray opacity of skin and subcutaneous tissue, with some hemorrhage; marked œdema extending from this spot to right axilla and backward to right groin.

Microscopically, a few bacilli corresponding in size to Loeffler bacilli found in the œdematous fluid. Microscopic examination of blood, lungs, liver, kidneys and spleen negative. Kidneys swollen, opaque and grayish in sections; extensive swelling and degeneration of epithelium in convoluted tubes; a few casts seen in the tubules. From œdematous fluid cultures in two serum tubes showed on following day numerous small colonies of a bacillus corresponding to Loeffler bacillus with well marked involution forms, staining well by Gram's method. Cultures from blood, spleen, lungs, liver and kidneys, all remained sterile.

The remaining cases, except a few of special interest, are briefly summarised in a table, as they were for the most part practically repetitions of the one given above. One case was of special interest and occurred in the private practice of Dr. Major. In this case, which I saw for the first time on the seventh day,

the membrane had nearly all gone, and on the first examination no Loeffler bacilli were found, although two agar tubes and two serum tubes, as well as three Petri dishes, were all seeded from a piece of membrane. In this case an oïdium appeared strongly resembling that of thrush, and I considered the case to be thrush complicated with staphylococcus infection. Dr. Major stated, however, that at the onset the condition was unmistakably that of diphtheria, and the correctness of his diagnosis was proved some days later by the onset of severe and persistent paralysis of the palate. In the interval I had obtained a fresh piece of membrane which yielded two colonies of the Loeffler bacillus. This case shows that a negative result is of no diagnostic value when the membrane is clearing, even when a fairly exhaustive examination has apparently been made. In another case I found an oïdium growth, readily distinguishable, however, from thrush, associated with a large number of Loeffler bacilli.

The uncertainty of examinations made at a late period in cases going on to recovery was shown in another case, for the opportunity of examining which I am indebted to Dr. W. S. England. In this case I saw the patient on the seventh day. A distinctly membranous exudation had been present, returning within twenty-four hours after being scraped off, but always confined to the tonsils. In this case smear cultures on five glycerine agar tubes failed to show any bacilli, the seeding being done directly from the membrane in the throat. In this case a tiny particle of membrane which had been obtained at the time of examination was seeded on serum a week later, and yielded two colonies corresponding to the Loeffler bacilli, one of which was tested and found to kill a guinea-pig in the typical manner.

In two cases I had great difficulty in obtaining suitable material for examination, owing to the affection being confined to the posterior nares, and where a prolonged local examination seemed unjustifiable owing to the profound exhaustion of the patient. In these cases I made cultures daily from the nasal discharge for several days, but without in any case obtaining the Loeffler colonies. In one of these cases (Case A 2) the nurse afterward obtained for me a small fragment of membrane

from which I obtained colonies having the characteristic culture appearances, and which killed a guinea-pig in the typical manner. In the other case (Case B 2), staphylococcus aureus and citreus were present in enormous number, together with a few streptococci, but no Loeffler bacilli were found.

In a fatal case (Case A 9), where an autopsy was performed by Dr. Finley, who kindly sent me the respiratory organs for examination, the larynx and trachea showed an extensive sheathing of diphtheritic membrane extending down to the main bronchi. Large numbers of the Loeffler bacilli were obtained from this membrane, and an area of pneumonia from the lung showed a small number of the bacilli associated with a large number of small diplococci. No streptococci were found.

In an anomalous case already cited, brought to my notice by Dr. H. S. Birkett (Case B 5), an extensive soft, yellowish membrane covered the posterior nares and extended over the epiglottis and into the larynx. This membrane could be readily removed without causing bleeding, but tended to recur. The general health of the child was unaffected. Cultures on agar yielded an abundant growth of a single bacillus form, forming prominent yellow white surface colonies. Inoculated into the conjunctiva of rabbits, no effect was produced, and I was inclined at the time to regard the bacteria as being possibly the pseudo-bacillus, but on re-investigating the cultures some three months later I found that they gave the typical Loeffler colonies on serum, with an abundant invisible growth on potatoes, and showed on both these media most characteristic involution forms. Inoculated into a guinea-pig, this organism showed a high degree of toxic virulence, killing the animal in thirty-six hours. The autopsy on this animal showed an opaque hæmorrhagic, reddish-gray indurated area at the spot of inoculation, surrounded by a zone of serous œdema. Cultures from the organs and from the serous exudation remained sterile, but typical Loeffler colonies were obtained from the circumscribed hæmorrhagic patch at the site of inoculation. These colonies, on being transplanted on agar, gave only the characteristic, flat, compact, concentric surface growth of the Loeffler organism, and not the abundant

prominent growth of the original tubes. For this reason it appears probable that the original colonies were impure, containing both the Loeffler colonies and some other form, which latter was subsequently eliminated in passing through the serum culture.

In cases which were examined at an early stage the Loeffler bacilli were found almost in pure culture. In most cases the staphylococcus aureus was found, but it was usually scanty. The colonies closely resemble those of the Loeffler bacilli for the first day, but can be distinguished at the end of forty-eight hours. Streptococci were seldom met with, and then only in isolated colonies, the scarcity of this organism being contrary to what I had been led to expect from a study of the literature. In the anomalous or doubtful cases, on the other hand, either staphylococcus and streptococcus, or both together, were present abundantly in all instances. The influence of these pyogenic bacteria on the course of true diphtheria is a point greatly needing investigation.

In cases examined during the period when the membrane had begun to soften—that is to say, after the third day in ordinary mild cases—the falling off in the number of diphtheria bacilli was most marked. This did not seem to be due here to the antagonistic action of saprophytic bacteria, as has been commonly assumed, since, as a rule, the cultures made in late stages in cases going on to recovery remained perfectly sterile, except for the few scattered colonies of Loeffler bacilli. This result I attribute to the inhibitory action of the local disinfectant applications—in most of the cases a spray of peroxide of hydrogen was employed—but I have made no experiments to determine this point.

A matter of considerable interest is the question whether this method of examination will ever come into general use, and if such be the case, what class of practitioners could carry it out to the best advantage. This point is considered by Prof. Welch of Baltimore in an address delivered before the ninety-third annual session of the Medical and Chirurgical State Faculty of Maryland in April, 1891 (*Medical News*, May 16, 1891). In

this addition, which by a masterly treatment of the facts places the prophylaxis and treatment of diphtheria on a broad scientific basis, Professor Welch does not think that the hopes of Roux and Yersin, that the method may yield good results in the hands of unskilled persons, are likely to be realised. He thinks that an elementary training in bacteriology is needed in those carrying it out, and suggests that as elementary courses on bacteriology have now become so common that it will not be long before most communities will possess at least one person capable of doing the work satisfactorily. My own experience has shown me that doubtful cases, which are those in which the value of the method should be best shown, the chief difficulty is in obtaining suitable material for examination are of two kinds—first, those where the condition resembles tonsillitis and the exudation is confined to the tonsils; and, second, those where the local disease is situated in some part of the respiratory tract not readily examined, or where the severity of the constitutional symptoms renders a prolonged examination difficult. As the procuring of a small piece of the false membrane at the earliest period possible is the *sine qua non*, and to do that in this latter class of cases requires a special manipulative skill only possessed by a skilled laryngologist, the task will properly fall to this individual. The aid of a throat specialist seems indispensable in cases where the local examination presents much difficulty. Possessed of suitable material, the best results in the further examination would certainly be obtained in a properly equipped laboratory.

In the cases where the confusion arises from the membrane being confined to the tonsils, the case is much more simple, and there is less need of the services of the laryngologist; all that is required being to detach a small piece of the exudation, wrap it in paper, and send it to some laboratory for examination.

Considering the gravity of the interests at stake in the prompt recognition and isolation of cases of diphtheria, one would naturally suppose that a method which enabled a positive diagnosis to be made within twenty-four hours, in cases seen during the first days of the illness, would be welcomed eagerly by the profession. That this has not happened is due probably in great part

to the wise precaution of treating all doubtful cases as if they were cases of diphtheria, and possibly, too, in some degree to a tendency to pride ourselves upon our sagacity, valuing the result of a clever guess more than that obtained by a less brilliant, though more certain, method. As a matter of experience, a large proportion of the doubtful cases, especially the tonsillitis group, declare themselves to be one thing or the other by the time the doctor makes his visit on the following day.

In conclusion, it may be stated :

(1) That in almost all cases where strong clinical grounds exist for the diagnosis of diphtheria, the bacteriological examination has shown the almost invariable presence of the malignant Loeffler bacilli.

(2) That, excepting in connection with scarletina, measles or erysipelas, the number of cases of diphtheritic sore throat due to other causes is very small.

(3) That in doubtful cases the accuracy of the method depends chiefly upon obtaining suitable material at an early stage of the disease.

(4) That the method is not of much service in doubtful cases where the difficulty is due to the infection occurring in localities difficult to examine without skilled manipulation, unless suitable material is obtained for examination.

SUMMARY OF METHOD FOR DETECTING LÖEFLER BACILLI.

Microscopical Examination.—Stain a cover-glass smeared by a bit of membrane with any aniline dye. The bacilli are arranged in small clumps, and are short, thick rods, about same length as tubercle bacilli, but much thicker; numerous beaded and drumstick shapes met with—in solution forms. Gram's staining method can be employed.

Cultures.—Can be made direct from membrane in throat or from small bit of membrane folded dry in clean paper. No special antiseptic precautions necessary. Touch or scrape membrane with a sterilized platinum needle and draw it in parallel streaks over the surface of a serum tube, using two or more successive tubes before re-infecting the needle. Keep the tubes at body temperature. In 20-24 hours the Loeffler bacilli appear as small grayish-white points, size of pin-heads, showing under the microscope the characteristic appearances of the bacilli in the original membrane.

Diagnosis.—(a) Other bacilli do not form visible colonies at twenty-four hours. (b) Staphylococcus colonies resemble those of Loeffler bacilli to naked eye appearance, but recognized on microscopic examination. (c) Pseudo-diphtheritic bacilli have microscopic and culture characters of the Loeffler bacilli, but have no pathogenic properties.

Pathogenesis.—Subcutaneous inoculation of guinea-pig kills in two to five days, with hæmorrhagic necrosis and œdema at site of inoculation. The bacilli can be recognised microscopically and by culture near spot of inoculation, but blood and viscera give negative results. Disseminated parenchymatous degeneration of liver and kidneys.

The following is a tabular analysis of the cases, divided into two groups:—

GROUP A.—Cases evidently diphtheria.

Number of cases examined, 9. Result: Positive 8; Negative, 1.

No.	Name.	Sex.	Age	Service.	Course.	Result of Examination.	Remarks.
1	M. L.	F	21	M. G. Hospital.	Recovery	Typical.* Oidium also found.	Began as Tonsillitis,
2	L. E.	M	2	Do.	Death.	Typical.	Nasal diphtheria. Bacilli in bit of membrane. None in nasal secretion.
3	Mrs. T.	F	28	Dr. England.	Recovery	Two colonies only.	
4	W. P.	M	16	M. G. Hospital.	Do.	No Loeffler bacilli; abundant staphylococcus pyogenes aureus.	Rapid disappearance of membrane, (nasal voice).
5	L. M.	F	10	Dr. Major.	Do.	Only two colonies present Oidium	Marked diphtherial paralysis.
6	F. P.	M	8	M. G. Hospital.	Do.	Typical.	
7	E. G.	F	10	Do.	Do.	Typical.	
8	L. P.	M	13	Do.	Do.	Typical.	
9	M. G.	F	4	Do.	Death.	Typical.	

* In cases marked "typical" a large number of the virulent Loeffler bacilli were found.

GROUP B.—Anomalous cases.*

Number of cases, 5. Result: Positive, 1; Negative, 4.

1	O. P.	F	20	M. G. Hospital.	Recovery	Negative.	Exudate confined to Tonsils.
2	C. W.	F	24	Do.	Do.	Negative.	Nasal diphtheria? Nasal voice. Local examination unsatisfactory.
3	A. C.	M	1	Do.	Do.	Negative.	Local examination unsatisfactory.
5	W. J.	M	8	Do.	Do.	Negative.	Scarletina.
6	S.	M	8	Dr. Birkett.	Do.	Positive.	

* Cases 1, 2 and 3 of this series were placed in the diphtheria ward of the Montreal General Hospital. Several cases of follicular tonsillitis were also examined, always with negative results.

