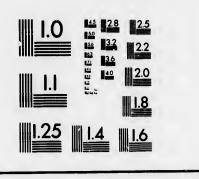


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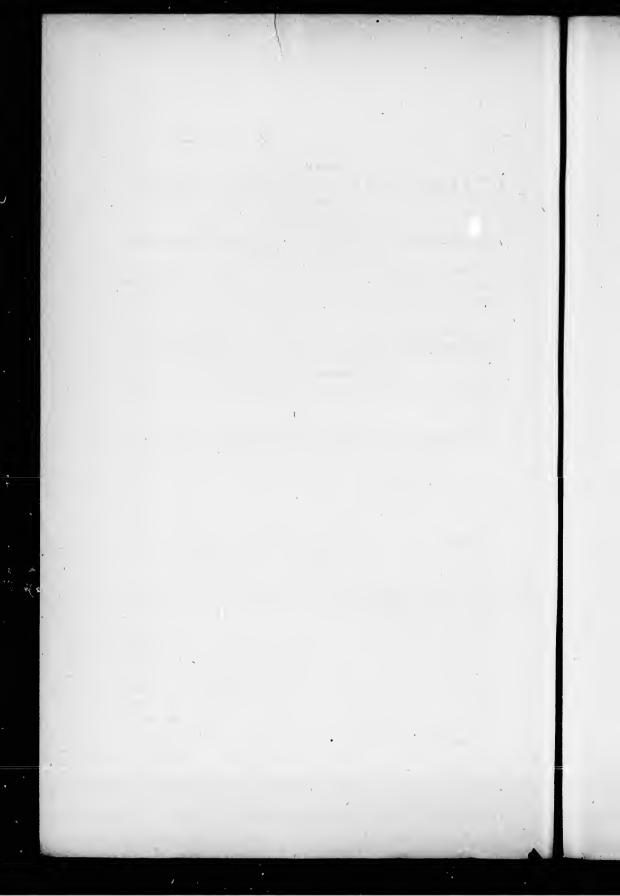
REPORT ON A YEAR'S WORK IN BACTERIOLOGICAL DIAGNOSIS OF DIPHTHERIA.

BY

WYATT JOHNSTON, M.D.,

Bacteriologist to the Board of Health for the Province of Quebec : Pathologist to the Montreal General Hospital.

(Reprinted from the Montreal Medical Journal, July, 1896.)



REPORT ON

A YEAR'S WORK IN BACTERIOLOGICAL DIAGNOSIS OF DIPHTHERIA.¹

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WYATT JOHNSTON, M.D.,

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Until a year ago no considerable number of bacteriological examinations had been made of diphtheria cases in Montreal. During the twelve months ending March 31st, 1896, there were sent from the two laboratories of which I have charge, 998 samples of exudation examined as to the presence of the diphtheria bacilli. Of those 528 were examined at the Provincial Board Laboratory, including samples from all cases admitted into the diphtheria wards of the Catholic section of the Civic Infectious Hospital, and 470 at the General Hospital Pathological Laboratory, including samples from each case admitted as diphtheria into the Protestant section of the Civic Hospital.

The examination was made by me personally in 729 cases. The remainder were examined by either Dr. J. E. Laberge (128), Dr. W. H. Jamieson (89), Dr. J. A. Williams (5), or the late Dr. E. P. Williams (47), who at one time or another kindly undertook my work during my illness or absence.

Methods—The methods followed were in the main those adopted by the New York Board of Health, which was the first sanitary body to introduce a system of wholesale bacteriological examination in diagnosing diphtheria. The fact that this precedent has been followed within two years by the establishment of similar services in many large American and European towns, and that in no case have any very important modifications or improvements been introduced speaks volumes for the efficacy of the system originated by Drs. H. M. Biggs and W. H. Park.

The Board of Health of the Province of Quebec considered that cheap card-board boxes which could be destroyed when once used would be preferable to the more elaborate outfits in use elsewhere and in view of the greater uniformity of results obtained the Board has only issued outfits with swabs, the cultures being made in the laboratory. Suitable blanks for filling in reports are sent out

¹ Read before the Montreal Medico-Chirurgical Society, April 3rd, 1896.

with each outfit and these outfits are left at pharmacies conveniently situated for all parts of the city. The fact that the service was a provincial and not a municipal one made it necessary that we should not attempt to do more for Montreal than we were prepared to do for the entire province, and therefore the enforcing of quarantine and local notification arrangements are not attempted, nor is a daily collection made from the depots, as should certainly be done in the case of a municipal diphtheria service.

Unfortunately the absence of permission to use the mails for transmitting samples of throat exudate has made it difficult to get samples to the laboratory in time for sending a report on the following day, and this has often prevented physicians from availing themselves of the facilities offered by the Provincial Board of Health.

Preparation of the medium¹—I have employed throughout Læffler serum, made by adding ½ volume of alkaline, 1 p.c. peptone, 1 p.c. glucose beef broth to ox-blood serum, simultaneously sterilized and coagulated in a water oven. (Hueppes method.) This medium gives a good growth at the end of 12 to 14 hours. The trouble and delay of frequent visits to the abattoir has been avoided by preparing large quantities of the serum at a time, adding with the glucose bouillon as a preservative, 1 to 2 per cent. chloroform and keeping it in self-sealing preserve jars until required for filling the tubes. I have used this method (suggested originally by Koch) since 1892 with very satisfactory results, and have still a reserve supply of serum kept by this means in ordinary corked vials since 1892, which was recently tested and found to yield a satisfactory medium.

It may be well to mention that if the serum contains much blood this will form a compact layer over the chloroform in the bottom hence it is better to shake the jar occasionally during the first few days.

By wrapping tin-foil round the tops of the tubes and dipping them into paraffin, they may be kept indefinitely without drying up. The tin-foil prevents the paraffin from entering the cotton wool. I owe the suggestion to Dr. Adami.

Method of taking samples—A small galvanized iron rod with a piece of cotton wool is fwisted round the end sterilized and placed in a sterilized tube, enclosed in a card-board box, with directions for use, forms the outfit of the Provincial Laboratory. In the case of the General Hospital Laboratory, a serum tube, for making the culture at once, is sent with the swab.

¹ Hard boiled eggs have been used as a culture medium from time to time when we happened to be short of serum in the laboratory, but I found they require more time and skill in order to properly make culture than is the case with the serum sterilized in tubes.

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It is better not only to rub the surface of the exudate with the swab, but also to pass the swab along under one edge of the exudate MacCollum), as bacilli may sometimes be met with in this situation after they have disappeared from the surface. In cases of laryngeal or nasal diphtheria, the bacilli are usually present in the mucus on the posterior wall of the pharynx. In case of negative results innoculation should be made, if possible, with pieces of membrane. It must be borne in mind that in some eases of diphtheritic croup, the bacilli may only appear late in the disease.

The use of ordinary cotton wool (as recommended by Shuttleworth) is preferable to absorbent cotton. The swabs should be thoroughly sterilized before use.

The result of the examination is communicated by mail, and also by telephone if desired, by noon on the day following the receipt of the swab at the laboratory.

In the cases of an anomalous growth or none at all on the serum, I have been in the habit of examining the swab microscopically, but my experience is in accord with that of Park, that the routine examination of the swab in all cases seldom gives additional information or enables a certain conclusion to be arrived at earlier than by the ordinary culture methods. The direct microscopic examination is a work which falls within the province of the clinician rather than of the laboratory bacteriologist.

I have tabulated 882 samples received during the twelve months ending March 1, 1896. Of these 572, or 64 per cent., were primary examinations or first samples and 310 secondary samples examined subsequently, either to determine when the throat became clear from bacilli in the genuine cases, or for the further study of doubtful ones.

Of 572 primary samples, 321 (56 per cent.) gave positive results; 189 (33 per cent.) negative results, and 52 (11 per cent.) doubtful results. So that we had to deal presumably with rather more than 321 cases of genuine diphtheria.

The relative frequency of mixed infection is shown from the following table of primary samples:

66	66	in pure culture	43]	per cent.
66		with streptococci	15	44
"		With staphylococci and streptococci	12	66
"		staphylococci alone	10	"
				"
Short bacilli	i and othe	r combinations	4	44
			100	

Unfortunately I had no opportunities for determining the relative mortality of the different associations and my information as to the relative benefits from antitoxin in each is too fragmentary to have any value.

Persistence of Infection in the Throat—Of 310 re-examinations or secondary cultures, made to see whether the bacilli had disappeared from the throat in the later stages or in the event of a doubtful result in a primary culture, 145 or 47 per cent. gave positive results. Among these there was only one case (E. C.) where the bacilli were missed at the first examination and found subsequently. This was a case of laryngeal diphtheria following pharyngeal, where the case was first examined bacteriologically on the ninth day of the illness. As a rule the bacilli become scanty in a membrane that has been formed more than four days, though in some cases they were abundantly present for several weeks after the disappearance of the membrane, and in two cases virulent bacilli were still present four weeks after the throat had been quite free from membrane.

On the other hand, they were absent in about one-half the cases by the third day after the disappearance of the membrane, corresponding with the statement made by Park. Under these circumstances the advantages of a quarantine regulated by bacteriological examinations

over one with an arbitrary time limit is obvious.

In cases treated with antitoxin the bacilli persisted as long in the throat as those treated without antitoxin. Personally, from what is known of the biology of the diphtheria bacillus and especially its tendency to die out or fail to increase in the presence of very weak acids, I should favour some mild treatment by lozenges or confection containing a weak organic acid, such as citric acid, by which an acid condition of the fauces could be kept up would be the most promising way of getting rid of the bacilli, but I have had no opportunity of testing this. I notice also that no note is made of the reaction of the fauces with reference to the persistence of bacilli.

Significance of Negative Results—With regard to the significance of a negative result, it depends altogether upon the stage at which the sample is taken. In case of negative results we have always requested the sending of secondary samples if anything in the course of the case tended to show that the case was really diphtheria. The result in these cases was almost universally negative. Out of 189 cases with negative results I have been able to hear of but two cases, where a visible membrane gave negative bacteriological results in the early stage, being followed by diphtherial paralysis.

The chief difficulty to the bacteriologist in interpreting these negative results is the absence of certain information that the sample has been properly taken. The occurrence of diphtherial paralysis

I regard as conclusive evidence that the case was really diphtheria. When the clinical diagnosis is positive I think that even after negative bacterial results, samples should be taken at intervals of a day or two in case of a re-infection. In any case, a negative result shows that the exudate is not at the time in a state likely to spread infection.

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It was not found, as a rule, that the taking of duplicate samples at the same time gave any additional information, though in one case bacilli were absent in one sample and present in a duplicate taken at the same time. Additional samples taken after an interval of 12 to 24 hours were more often of service.

In any case where the patient has been ill for four or five days the absence of bacilli in the cultures, even from visible membrane, should not be considered as proving that the case is not one of diphtheria. When the sample is taken from cases with no visible membrane the negative results are less trustworthy, but as a rule in cases of croup the posterior wall of the pharynx gives an abundant growth of diphtheria bacilli. Some throat swabs taken from fatal cases of croup not diagnosed during life were found to give positive results, a method which might be made to yield valuable statistical information in a properly managed system of death certification. The fact that so-called simple croup is credited with causing in Montreal double the number of deaths due to typhoid should make this question worth investigation.

Doubtful Results—In 11 per cent. of the primary cases the results of the first culture were not decisive; of these 1 per cent. showed entire absence of growth, due to the use of antiseptic applications shortly before taking the sample. Sometimes organisms, apparently characteristic Klebs-Læffler bacilli, were present in such small numbers that they did not correspond with the known tendency of diphtheritic bacilli to rapid growth as compared with other organisms. In such cases a second culture was uniformly asked for and obtained, but it was just as uniformly negative, except when obtained in the late stages. If one is sure that the sample, properly taken from visible membrane in an early stage, gives negative results, there is very little danger of the case being diphtheria. With regard to the late cases in which few bacilli are met with, it is likely at all events that the danger of infection will be as slight, so long as the bacilli are scanty in the exudate.

The routine advice has been to keep such cases isolated and send further samples. In case they are exposed to danger of infection from undoubted cases of diphtheria, or removed to an infectious

hospital, a small dose of antitoxin would confer immunity. Thispower of immunization offsets to some extent the lack of any proper isolating wards for observation of doubiful cases in our contagious hospital. With a scanty growth of suspicious looking bacilli, I am inclined now to look upon the case as probably not diphtheria, if in an early, or probably not highly infectious if in a later stage. As severe angina so often proves to be a manifestation or complication of scarlatina or some other exanthem, I favour isolation in a fever hospital, if not obtainable otherwise, as being the safest plan for the public safety. The clinical course of these cases, however, often shows them to be tonsillitis by the end of 24 or 48 hours. 'My experience of such cases has been decidedly that they are not infectious. They usually improve suddenly if left to themselves, and so if a dose of antitoxin happens to be taken this may create the impression that the case was really diphtheria. The absence from the cultures of a sufficient number of streptococci to explain the condition of pseudo-membranous angina is suspicious of diphtheria, as most of the non-diphtheritic anginas are due to streptococci. As I have information about several cases, not diphtheria, benefited by antitoxin, I think its employment in all doubtful cases should be the rule if the symptoms are grave, especially if there are evidences of croup. 'Our experience in the Hospital laboratory has been that an unusual number of doubtful results were found in connection with private cases. It was the rule in such cases to find a considerable number of suspicious looking bacilli in many cases which we did not consider to be diphtheria. The only explanation which suggests itself to me is, that the samples were taken in these cases with unusual care, and an unusually liberal amount of the exudate was smeared on the culture media, and that under these circumstances a certain amount of growth in the natural secretions of the pharynx may have been possible with germs which do not grow on Leeffler serum. Sometimes, where the growth was scanty, portions of the exudate itself would be removed from the serum in examining the culture. The presence of bacilli in the exudate, which do not grow on the media, is against the probability of diphtheria.

An abundant growth is sometimes found of a bacillus which does not have the typical characters of Klebs Leeffler bacilli.

These cultures constituted between 1 and 2 per cent. of all the samples examined and were extremely puzzling. They usually occur in groups, and are associated with contact or exposure to genuine diphtheria. None of them were fatal in my experience, though two or three were followed by pharyngeal paralysis. The membrane

formation was described as not being typical but usually thick and pultaceous, and in some cases no distinct membrane was formed.

In one such case, the rather large organism met with grew on potatoes as a distinct white colony, excluding it from the category of Klebs-Læffler bacilli. It killed guinea pigs by septicæmia without the characteristic hæmorrhagic infiltration of the diphtheria bacilli.

In the case of genuine diphtheria bacilli, a certain proportion of the rods stain intensely, almost black, with warm carbol fuchsin. In the case of the pseudo-forms this was not the case, the staining being more uniform and much fainter.

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In another case an organism forming long rods was obtained in pure culture and killed guinea pigs in the typical manner with the characteristic lesions.

In a third case, without any diphtheritic membrane, an organism was met with which was twice as long and thicker than the Klebs-Læffler bacillus in the first culture, but on making sub-cultures in successive generations became typical, and later on showed typical growth from secondary throat cultures.

This tendency of bacilli, when in considerable amount in the first cultures, to revert to the well-known Klebs-Læffler type in subcultures and their tendency to show virulence to guinea pigs, has made it hard to decide that any bacillus is not Klebs-Læffler bacillus if it is found in abundance in a primary culture, no matter how far it may be removed from the orthodox standard morphologically.

The most satisfactory mode of procedure is, of course, the inoculation of guinea pigs, but to do this properly involves delay. In the first place, the original throat cultures are nearly always too thickly sown to allow of the immediate isolation of pure cultures without preliminary separation upon surface of serum tubes, which means usually a delay of a day. When a pure culture is obtained conclusive results can only be assured by inoculation of a bouillon culture, which means a delay of one or two days more. Finally, if the full degree of virulence is not present, the animal may take two or three days to die. In any case, as pointed out by Roux and Yersin and by Park, the fact that one colony grown from a sample is not virulent, does not show that another of the same bacillus from the same case may not be, as the virulence of different colonies, even in the same case, has been shown to vary greatly. The formation of acid or alkali can be more readily determined, but also requires isolation of pure cultures in order to be used.

The delay and trouble attendant on this test by inoculation contrasts very unfavourably with the convenience and rapidity of the rest of the technique for quarantine purposes. I have obtained more satis-

factory results from simply making secondary cultures; it will be found either that the bacilli are absent from the cultures or that more typical forms are met with.

Where there is definite growth of bacilli from the throat, the case should be provisionally regarded as one of diphtheria until shown to be otherwise, whether the clinical symptoms and the morphological characters are typical or not, as the tendency to variability is far more distinctive of the diphtheria bacillus than any one of the forms in which it occurs.

Visible growths on acid petato, or motility and formation of alkali are sufficient to characterize any organism showing them as something else than the Læffler bacilli.

Significance of Positive Results.—When a typical heavy growth of a bacillus, corresponding in appearance with the Klebs bacillus, is met with, there need be no hesitation in declaring that the conditions for diphtheritic infection are present, whether the clinical symptoms correspond or not, and er there is visible membrane or not.

Of the 293 prima ples examined at the Provincial Board of Health Laboratory, 40, or 13 per cent., were from cases diagnosed clinically as follicular tonsillitis, quite apart from a number more where the diagnosis was stated to be possibly or probably diphtheria. Of these 43 cases diagnosed as tonsillitis, 19, cr 45 per cent., were shown to be diphtheria. This result was confirmed by inoculation experiments in a number of my earlier cases, the result being uniformly positive in every case where it was tried. This experience tends to shake one's confidence considerably as to the efficiency of the diagnosis of tonsillitis from diphtheria without making cultures. As to the recognition of diphtheria from tonsillitis, of 293 primary samples sent to the Provincial Bacteriological Laboratory, only 173, or 59 per cent., gave positive results, and of 279 primary specimens sent to the pathological laboratory of the Montreal General Hospital only 148, or 53 per cent, gave positive results. Possibly many of these were only sent as an additional precaution in cases clinically considered as tonsillitis, but we found that cases called follicular tonsillitis were really diphtheria 45 times out of 100.

We may assume that when a patient is sent to a diphtheria ward in an infectious hospital some good grounds exist for diagnosing the case, but in the case of the Catholic division of the infectious hospital of 73 primary samples from cases sent as diphtheria, only 46, or 66 per cent., showed the presence of diphtheria bacilli, while 25 or over showed none.

In the case of the Protestant section of 92 primary samples, 81, or

87 per cent., showed the presence of diphtheria bacilli and 11, or 13 per cent., showed none. The difference between the results of the two sections appears to be due to the fact that a preliminary bacteriological examination was made in a large number of patients at the Montreal General Hospital before forwarding cases recommended for admission as diphtheria.

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From the above, I have omitted from the negative result in each division, two patients admitted in the paralytic stage of undoubted attacks of diphtheria, but from whose throats the bacilli could no longer be obtained.

The results go to show the advisability of making, whenever possible, preliminary bacteriological examinations of cases before sending them to the infectious hospitals, and also of providing properly isolated observation wards in the infectious hospitals for the reception of doubtful cases. A precautionary immunising dose of serun will considerably lessen the danger of an infection resulting from mistakes in diagnosis, but it will not wholly guard against it as I have met with several instances of diphtheria infection, confirmed by bacterial examination, among persons supposed to be protected by doses of the antitoxin.

Accuracy of Various Methods of Diagnosis.—The delicacy of the positive clinical diagnosis of diphtheria has been seen in our 572 cases to be somewhat over 60 per cent. The bacteriological diagnosis has, in my experience, shown an accuracy of somewhat over 90 per cent., that is to say, a doubtful opinion had to be given in one case out of every ten primary samples examined, and the subsequent course of the cases usually showed them not to have been diphtheria. The chief difficulty in giving a diagnosis is the uncertainty from not having personally made the cultures.* I found a greater uniformity of the results in the Provincial Laboratory where all the tubes are inoculated by one attendant who strictly followed my instructions, than in the hospital laboratory where they were made by various physicians.

Probably had specimens been indiscriminately sent me in all cases the percentage of genuine cases would have been greater, but naturally one had a preponderance of the obscure cases as, by some physicians, samples were only sent from those which were puzzling.

The positive results were equally frequent in the case of the hospital cases, where cultures were made at once on serum and forwarded to the laboratory, and in those of the Provincial Laboratory,

^{*} In one case shown by autopsy to be laryngeal diphthcria a physician inoculated a tube which gave negative results. A few hours later in performing a tracheotomy on the same case he accidentally inoculated his own hand, producing a sloughing wound from which we obtained a pure culture of Klebs Læffler bacilli.

where the swabs were sent to the laboratory and the culture made as a rule only after several hours delay.

Diphtheria Bacilli in the Throat of Apparently Healthy Persons—The occasional occurrence of bacilli, like the diphtheria bacillus, so-called pseudo-diphtheria bacilli, in the throat of apparently healthy persons appears to have received an undue amount of attention with reference to its practical bearing upon the diagnosis of diphtheria. These bacilli have very seldom been met with in the overwhelming numbers which characterize the early stages of diphtherial infection, and therefore are not likely to give rise to serious errors of diagnosis in cases examined early.

On the other hand, it has been abundantly demonstrated that the virulent diphtheria bacilli may exist in large numbers in the throat of persons who are apparently healthy and who certainly do not present any of the clinical features of diphtheria, and this important discovery viewed in its true light should materally influence one's course in dealing with outbreaks of this disease in households or public institutions such as schools, hospitals and asylums.

Instead of isolating and examining bacteriologically only those cases which have sore throat, all the throats in the institution or household should be examined bacteriologically and those persons from whom a culture of diphtheria bacilli is obtained should be isolated and quarantined, whether they are ill or not, until no bacilli are found in the throat cultures.

This is probably the explanation of the difficulty often experienced in preventing the spread of diplitheria in households or public institutions, even after all persons actually ill have been rigidly quarantined.

Attention to this mode of infection by the New York Board of Health has led to most satisfactory quarantine results, and Asser, of Copenhagen, has recently reported an outbreak in a cavalry regiment which continued to spread after all the sick persons had been isolated and thorough disinfection performed, and was only controlled when 19 persons, apparently healthy, but whose throats furnished growths of diphtheria bacilli, were quarantined.

Use of Antitoxin.—The twelve months under consideration, cover pretty accurately well the first twelve months' experience with the use of antitoxin and therefore the first years in which a specific remedy was available for the treatment of this disease. It is of special interest to study the frequency of diphtheria in the city during this period and see what effect this new remedy, whose efficacy is undoubted, has had upon the diphtheria death rate.

From the records at the City Health Office, to which Dr. Laberge, City Health Officer, kindly gave me access, I find that during the period in question 849 cases of diphtheria and diphtheritic croup were reported at the health office, besides 83 cases reported as simple croup.

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The mortality from diphtheria and diphtheritic croup was 282, and in addition there were 100 deaths from so-called simple croup. Probably three-fourths of the cases of this latter disease were really diphtheria. In Montreal the term of "simple croup" was recently invented to cover obscure throat cases in which notification is permissible but not obligatory, in the hope that it may lead to cases being reported which would otherwise not be heard of. If the cases thus reported were examined bacteriologically valuable information would be obtained. The deaths registered under this heading, in spite of its harmless and reassuring sound, are twice as many as those occurring from typhoid fever in Montreal.

The total mortality for diphtheria and croup was thus in the neighbourhood of 400 for the twelve months under consideration and double that observed in any one year since 1888, the mortality from diphtheria and croup in the intervening years being as follows: 1888, 427; 1889, 200; 1890, 170; 1891, 78; 1892, 54; 1893, 65; 1894, 99; 1895, 419.

Without going into the proportion of the deaths in the different years to the increasing varying population of the city, I wish to call attention to the fact that during the first year of the antitoxin treatment, and in spite of the fact that this treatment was very generally carried out in private patients who could afford it and among hospital patients who get it without paying, and while special statistics show a decided lowering in the death rate, nevertheless in Montreal, twice as many persons died from diphtheria during 1895 as during any of the previous six years. This probably does not depend at all upon any better enforcement of notification in diphtheria cases, as it includes both diphtheria and croup.

My reason for emphasizing this is to show that the rigorous enforcement of the standard quarantine measures of isolation and disinfection is not rendered less necessary by the introduction of the new specific remedy. Indeed I consider that by converting a severe disease into a mild one the tendency of the patients to go about before the danger of infection had disappeared must be increased to an extent which offers a new element of danger, unless quarantine is very strictly enforced. Even physicians hardly realize the necessity of enforcing a tedious quarantine and isolation for what has apparently been trivial illness which improved promptly under antitoxin.

Another matter which calls for notice is the practice of withholding antitoxin until the diagnosis of diphtheria is clear. Probably a large proportion of the deaths from diphtheria arose from delay in the use of this remedy. One would think it better that the remedy should be given ninety nine times to persons not having diphtheria than to omit it in one genuine case.

I was often told that a report was wanted in order to determine whether it is necessary to use the antitoxin or not. The invariable answer has been not to delay the administration of antitoxin for a bacteriological report if the case is at all urgent. Although the coincidence of the discovery of this remedy has led to an increased interest in the diagnosis of diphtheria, the unavoidable delay of 15 to 24 hours makes it inadvisable to delay a preliminary "protective" dose of the remedy until the diagnosis has been confirmed. In any case, observations have shown marked benefit in certain forms of angina solely due to staphylococci and streptococci when diphtheria bacilli were found to be absent.

Conclusions.—1. The culture method enables a positive diagnosis to be made in 90 per cent. of all cases of diphtheria when seen early.

2. The significance attaching to a negative result depends entirely upon the length of time which has elapsed since the onset of the disease, and the absence of bacilli from a case which has lasted often four or five days does not prove that it is not diphtheria; in any case where the course of disease makes it likely to be diphtheria, repeated re-examination should be made.

3. In severe cases of suspicious angina, it is advisable not to delay the preliminary dose of antitoxin in order to learn the result of the

bacteriological examination.

4. The greatest value of the bacteriological examination is in determining the necessity and the duration of isolation and quarantine, and if cases continue to appear, the throats of all persons exposed to contagion should be examined whether they show signs of disease. or not. A swabbing to be taken post-mortem in all cases of death from croup.

5. The patients should not be released from quarantine and the final disinfection of the premises should not be done until the bacilli

have disappeared entirely from the affected part.

6. The bacilli have been shown to infect articles of clothing, furniture, etc., and these should be thoroughly disinfected, preferably by steam under pressure, and solutions of mercuric chloride. Furnigation by sulphur is unreliable in the majority of cases as commonly carried out.

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oioy aly 7. Cases showing a heavy growth of bacilli, on serum at 20 hours, not quite of the typical Klebs-Læffller type, should be regarded as suspicious and strictly isolated until their non-diphtherial nature is clear. In order to avoid the conflict between the opinions of the medical attendant and the bacteriologist, it is preferable when a case is diagnosed clinically as non-diphtheritic shows a growth of bacilli, that the further tests of acid production and pathogenes is to be applied, the medical attendant being informed of the suspicious nature of the case and of the necessity of isolation pending more thorough bacteriological study. This would remove a common cause of friction between the medical attendant and the Health Officer and lead to the more careful study of the doubtful cases.

8. In spite of the use of antitoxin and the great benefits resulting from it, the mortality of diphtheria has greatly increased in Montreal during the past year.

