THE TECHNIQUE OF DETECTING DIPHTHERIA BACILLUS CARRIERS AND ITS APPLICATION.

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Introduction.

The modern methods of preventing diphtheria include the detection and isolation of the virulent diphtheria bacillus carrier, the Schick testing of individuals to distinguish susceptibles from non-susceptibles, the active immunisation of susceptibles with toxin antitoxin mixture or one of its modifications, and occasionally, when circumstances seem to demand it, the administration of antitoxin to confer passive immunity sufficient to tide the individual over an epidemic. Theoretically it would be possible to stamp out diphtheria by adopting either wholesale isolation of carriers or active immunisation of all susceptibles. In practice, however, isolation of all carriers is impossible, except in limited communities such as schools, ships, asylums, jails and hospitals, where it may be attempted with a reasonable prospect of success. It is in fact in such communities that the method of isolating carriers is particularly applicable in order to check sporadic outbreaks of diphtheria, especially where the proportion of susceptible individuals to non-susceptibles is high, as for example in an infants' school. Its application to the control of epidemics and to lowering the endemic level of diphtheria incidence does not appear to have met with success, although some Public Health authorities pursue a policy of isolating carriers in addition to immunising susceptibles. It is obvious that the success or failure of the isolation method will depend very largely upon the quality of the technique employed in detecting the carriers. It is proposed in what follows to elaborate in some detail the technique which should be employed for this purpose and to consider its application in certain specific instances.

Despite the truly vast proportions of the literature on diphtheria and the many papers which have appeared from time to time dealing with the carrier rate, there does not appear to be a single full account of the methods to be employed in detecting carriers. There is no doubt that a trained bacteriologist would find little difficulty in evolving an ample and satisfactory technique for himself if called upon to do so, but for the many who have had no special training in this aspect of bacteriology there is no guide as to how the best results may be obtained. The report of the Bacteriological Committee of the Medical Research Council in 1923, which is regarded as being the standard work on the subject, does not refer to the technique, and I have been unable to find any account of it in the literature since then. Owing to this omission,

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unless a person called upon to make an examination for carriers has had some previous experience in that work he may be at a complete loss as to the best technique to employ. It is proposed to deal first with the methods which should be followed, to illustrate by examples the results which may be obtained with them, and then to consider some of their applications.

Preliminary observations.

For practical purposes diphtheria carriers are of three kinds, as classified anatomically: nose, throat and ear carriers. With regard to the last, the aural carrier state is almost always, if not always, associated with pathological conditions of the ear accompanied by a discharge and it is consequently only in the presence of such a discharge that ear swabs need be taken. Both nose and throat swabs, however, must be taken in every case, as the frequency of the nasal carrier is just as great as that of the throat carrier. This may be readily appreciated from the following table which gives the anatomical distribution of the organisms in 50 carriers found by me (1931) among 1000 Dublin school children, swabs being taken from both nose and throat.

Site	Positive cultures
Nose only	25
Throat only	18
Nose and throat	7
Total	50

The diphtheria bacillus is therefore to be sought in the nose and throat as a routine, and, when conditions warrant it, in the ear as well.

There are two types of diphtheria bacillus which may be found in these situations, either the virulent or the non-virulent type. Carriers of the non-virulent organism are not a menace to the community and do not require isolation and treatment as carriers of the virulent type do. The two types of carriers have, in consequence, to be distinguished from one another. This is done by isolating the organisms in pure culture and testing their toxin-producing powers by animal inoculation. The complete detection of the carrier, therefore, involves not only the successful culture of the diphtheria bacillus removed from the nose, throat or ear by means of a swab, and its identification by microscopical appearances, but also its isolation in pure culture and its final study by fermentation and virulence tests. If the isolation of carriers is to be both practicable and efficient their detection should involve as far as possible only one swabbing and the technique employed should therefore aim at securing 100 per cent. isolations of the diphtheria bacilli in the swabs.

From these preliminary considerations we may turn next to the details of the technique to be employed in order to achieve the desired results.

Technique of swabbing.

The requirements at the time of swabbing are a small table, a chair, an ample supply of swabs and spatulas, a sufficiency of slopes of Loeffler's serum

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for making primary cultures, a jar of 5 per cent. lysol and pen and paper for taking names. The slopes of Loeffler's serum should all be supplied with blank labels and three different coloured wools should be used to plug them to distinguish nose, throat and ear cultures. For example, an equal number of slopes with blue and white plugs may be provided for nose and throat cultures and a smaller number with green plugs for ear cultures.

For mass swabbings the swabs may be wrapped in white paper, a pair of swabs being enclosed in each wrapper. The requisite number of swabs to cover all purposes is then made into a package which is sterilised in the autoclave. This is much the most convenient way of carrying the swabs, as they occupy little space and their sterility is ensured, each pair being opened as it is required. For each throat swab a sterile spatula must be provided. It is best to wrap the spatulas in paper and to autoclave them also, opening each as it is required when the swab is being taken. The person taking the swabs should sit or stand at the table with the windows, open if circumstances permit, upon his right-hand side. On the table in front of him are placed the swabs, spatulas, tubes of Loeffler's serum and sheets of paper for taking names. He is approached from the left by the person to be swabbed and, having ascertained the name, enters it opposite the number of the examination. It greatly facilitates matters, particularly in dealing with young children, to have a teacher or other suitable person to take the names. The number of the examination is entered upon the labels on the serum tubes. As the swabs are taken they are well rubbed over the surface of the serum slopes, care being taken to assign each swab to a tube with wool of the correct colour. The jar of lysol is convenient to receive the used swabs and spatulas.

The position of the operator as described is worthy of note. He is situated between the person to be swabbed and the open window which is conducive to his comfort and lessens the risk of diphtheria bacilli being sprayed on to the various articles upon the table. Furthermore the light is to the best advantage and his right hand is in the most convenient position for writing and other movements.

In taking throat swabs care should be exercised that the swab comes in contact with as large a surface of the mucous membrane of the fauces and pharynx as possible, since there is no guide as to the situation in which the diphtheria bacilli may be found such as is present in cases of the disease. Similarly in taking nasal swabs, the swab should be inserted well into each nostril in turn and gently though firmly rubbed over the surface of the nasal mucous membrane. The same principles apply to the taking of aural swabs when they are necessary.

It is a great advantage to inoculate the swabs on to Loeffler's medium as soon as they have been taken. It prevents loss by drying and by outgrowth of the diphtheria bacilli by saprophytic organisms. Both these causes of loss are very considerable in their effects if some time must elapse before the swabs can be taken to the laboratory. It furthermore increases convenience

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without increasing the bulk of the material to be handled. It is more economical, decreases labour, saves time and makes negligible the risk of clerical errors. If the swabs were to be taken to the laboratory prior to inoculation it would involve the use of tubes just as large as those required for serum and would mean, in addition, the labelling of two sets of tubes, first the swabs and second the culture tubes, so increasing the risk of errors.

Technique of detection, isolation and identification of the diphtheria bacillus.

The use of Allison and Ayling's medium (1929) as a routine method of isolating the diphtheria bacillus in pure culture, from the primary growths on serum, is to be advocated. It may be well, therefore, to consider first the preparation of this medium and to discuss the results which it may be expected to give and to deal with its limitations and how they may be overcome. The conclusions with regard to its uses and limitations are based on 2000 inoculations made on the medium from primary serum growths of material taken from the nose and throat.

Allison and Ayling's medium is a modification of Douglas's trypsinised serum tellurite medium. For its preparation the following reagents are employed:

(i) A 2 per cent. agar of *p*H 8 with a basis of either Hartley's digest broth or 1 per cent. peptone broth containing meat extract or Lab. Lemco in the usual proportions.

(ii) Sterile trypsinised tellurite serum.

(iii) 10 per cent. copper sulphate solution in distilled water.

The agar may be conveniently sterilised and stored for use in milk bottles as supplied by United Glass Bottle Manufacturers, Ltd., 400 c.c. of agar being contained in each bottle. The trypsinised tellurite serum is prepared by adding for each 100 c.c. of horse serum 8 c.c. of Allen and Hanbury's Liquor Trypsinae Co. and 10 c.c. of a 2 per cent. aqueous solution of potassium tellurite. The serum is then filtered with aseptic precautions through a Seitz filter after which it may be transferred to sterile tubes and placed in the cold room where it will keep for several months. A large quantity may, therefore, be prepared at one time and stored until required. The tellurite causes a precipitate in the serum which appears to be due to the formation of the insoluble calcium tellurite. This produces a deposit in the tubes and they should consequently be warmed to 55° C. for a few minutes and well shaken up before use, to disperse the precipitate. The copper sulphate recommended by Allison (1930) is the B.D.H. copper sulphate A.R.

When the medium is required for use it may be made as follows. To every 100 c.c. of nutrient agar melted and cooled to 55° C., 10 c.c. of the sterile trypsinised tellurite serum and 0·5 c.c. of the 10 per cent. copper sulphate are added. After mixing thoroughly the medium is poured into sterile Petri dishes and, when set, is placed in the incubator to dry preparatory to use.

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The chief claims which have been made for this medium may be summarised briefly as follows:

(i) It prevents the growth of staphylococci and streptococci and allows the diphtheria bacillus and members of the diphtheroid group to grow unrestricted.

(ii) Spreading organisms of the *B. proteus* type, which are frequently present in the ear, are restricted in the limits of their growth to the formation of colonies.

(iii) The colony forms of the diphtheria bacillus and the diphtheroids are more characteristic than if copper sulphate is omitted from the medium, and in consequence colonies of the various members of the group may be distinguished from one another, leading to ready isolation of the diphtheria bacillus. The latter organism is said to form three distinct types of colony after 24 hours' incubation.

(*a*) A smooth glistening colony having a diameter of 0·75 to 2 mm. with a deep black central zone and a light grey peripheral belt sharply differentiated from the central area. The colony may or may not show a cup-shaped depression in the centre.

(*b*) Round glistening colonies about 0·5 mm. in diameter which are grey in colour.

(*c*) Dry, lustreless, flat colonies with an irregular margin and rough surface. They vary in size from 0·25 to 2·0 mm. and have a black centre with a greyish white peripheral zone.

(iv) A characteristic odour is said to be produced by the growth of the diphtheria bacillus.

For a full description of the colony forms of the diphtheria bacillus, Hofmann's bacillus and the other diphtheroids in addition to the various characteristics of the medium, the original articles should be consulted.

To deal with the first and second of the above claims, there is no doubt that the medium effectively suppresses or limits the growth of most organisms except those of the group to which the diphtheria bacillus belongs. In addition, such organisms as will grow, produce in general either white, yellow or brown colonies which cannot be confused with the colonies of the corynebacteria. It has been possible to confirm the authors' observations as to the nature of the unwanted organisms capable of growing on the medium, but it has been found in addition that there is a type of streptococcus which produces a flat white lustrous colony about 0·5 mm. in diameter which they do not mention and a number of moulds of various sorts. Although it is true to say that the majority of diphtheria bacilli are not inhibited in their growth by the presence of copper sulphate in the medium, none the less some are either inhibited for as long as 48 hours or else are completely suppressed. This suppression takes place even though the copper sulphate is present in only just sufficient quantity to achieve its object, namely, the inhibition of the organisms which by their luxuriant growth render the isolation of the diphtheria bacillus

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difficult or impossible. Allison (1930), in his later communication, shows that different brands of copper sulphate vary so greatly in their inhibiting power that some are five times as potent as others. This would seem to suggest that it is not the copper sulphate which is active but rather some impurity associated with it. The suppression of certain strains of diphtheria bacilli by the medium does not constitute a very serious drawback to its use, as this may be allowed for in cases where the diphtheria bacillus is known to be present by adopting another method of isolating the organism in conjunction with the special medium.

With regard to the third claim outlined above, the first type of colony described is undoubtedly the most characteristic type produced by the diphtheria bacillus and frequently can be recognised with a considerable degree of certainty. It is the type which should always arrest attention in picking colonies. As the authors mention, the second and third types of colony are much less frequently encountered. This may be seen by analysis of the results obtained with thirty-nine different strains of diphtheria bacilli on their medium. Of the thirty-nine strains thirty-two gave the first type of colony, six the second type, while none of the third type were found in the series. One strain gave a smooth, white colony about 1·5 mm. in diameter with no tendency to blacken. The authors do not mention the occurrence of this type of colony which is apparently uncommon. Hofmann's bacillus and other diphtheroids of common occurrence in the nose and throat are capable of producing all three types of colony described as characteristic of the diphtheria bacillus. It has repeatedly been found that when a colony conforming to one of the above types has been picked it yields a growth not of diphtheria bacilli but of a diphtheroid. It is necessary to sub-culture the organisms on serum in order to make certain that it is a diphtheria bacillus, which has been picked, owing to the fact that the microscopical morphology of the diphtheria bacillus is not reproduced on the special medium and it cannot therefore be recognised by staining a film from one of the colonies. It should, however, be stated that Hofmann's bacillus and the other diphtheroids frequently produce colony forms which are not given by the diphtheria bacillus, and these do not cause confusion. The net result is that in picking colonies one should select those which conform to one of the types given by the diphtheria bacillus, ignoring the others. By doing this a considerable number of the diphtheria group other than the diphtheria bacillus will be picked, but at least none of the diphtheria bacilli will be lost.

With regard to the fourth claim, the only odour noticeable from the plates on which diphtheria bacilli are grown is the garlic-like odour of certain tellurides. It is produced by the diphtheroids which have black colonies just as readily as by the diphtheria bacillus and does not serve as a guide.

To turn now to the application of the method to the isolation of the diphtheria bacilli present in the serum cultures made during the mass swabbings. On the day that these cultures are made the requisite number of plates

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of Allison and Ayling's medium to deal with them all are poured. They may be kept in the cold room over night and dried for a couple of hours on the next day before use. After the primary cultures have been incubated for 24 hours at 37° C., 1 c.c. of saline is added to each tube (a smaller amount being used when the growth is very light) and, after emulsifying the growth in the saline, films are made from the suspensions so obtained. The films are stained by one of the recognised methods of staining for diphtheria bacilli, the best and also the simplest being Loeffler's methylene blue. They are then examined microscopically for diphtheria bacilli.

No general rules can be given for the certain microscopical recognition of the diphtheria bacillus. It is a matter of considerable difficulty in many cases and facility of recognition can only be acquired by long and careful practice. In a given case it will, in all probability, be found that in some of the films from the cultures morphological diphtheria bacilli are present whereas in the majority of the films none can be seen. All the suspensions from the cultures, however, irrespective of whether diphtheria bacilli can be seen or not, should be inoculated heavily on the plates of the special medium and spread in the usual manner. Two or three loopfuls or more may be used for each plate.

The reason for plating all the suspensions is that Allison and Ayling's medium permits the ready isolation of diphtheria bacilli from suspensions of mixed organisms even when they are greatly outnumbered by these organisms and are present in such small numbers that they cannot be found in films from the suspensions. It may be argued that it is not worth while isolating the organisms from a carrier who gives such a light primary growth. Copeman, O'Brien, Eagleton and Glenny (1922) adopt the view that the risk from a carrier is proportional to the profuseness of the growth obtained from a swab on a serum slope. It should, however, be pointed out that even though the organisms are present in the nose and throat in considerable numbers it may well happen that the swab does not come in contact with a large number of them and consequently does not give a profuse growth. It must fall to every bacteriologist's lot to encounter bacteriologically established cases of diphtheria in which swabs yield little or no apparent growth of the diphtheria bacillus although it is abundantly present in the throat. As a matter of fact it is well known that carriers are notoriously intermittent in the profuseness of the growth which they yield, at one time giving a well-marked growth of the diphtheria bacillus, and at another no apparent growth at all. This intermittency is well shown by the experience of McCartney and Harvey (1928). It is consequently just as important to detect those carriers who show no apparent growth in the primary cultures on serum as those who provide a heavy growth.

Owing to the fact, already alluded to, that Allison and Ayling's medium suppresses a proportion of diphtheria bacilli, it is necessary, in order to ensure isolation of all the strains which are visible in the films from the primary cultures, to plate out the positive suspensions on an alternative medium.

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Blood agar has been found very satisfactory for this purpose after a little practice in recognition of the various colony forms which the diphtheria bacillus may produce on it. Alternatively plates of Douglas's medium or Loeffler's serum may be employed according to choice.

After 24 hours' incubation at 37° C. it is possible to pick suspicious colonies from Allison and Ayling's medium. It is wise to re-incubate the plates for a further 24 hours in cases where no suspicious colonies have appeared. The sub-cultures should be made on Loeffler's serum. It will be found that in most cases the special medium will give a culture of diphtheria bacilli when these organisms were visible in the corresponding primary cultures. If however they have been suppressed by the special medium they may be isolated from the alternative medium. It is quite possible to ensure the isolation of diphtheria bacilli in every case from cultures, in which they are known to be present, by adopting this technique. Suspicious colonies should also be picked on to Loeffler's serum from the plates of the special medium spread with the suspensions of the primary cultures not known to contain diphtheria bacilli.

The serum slopes are incubated at 37° C. for 18 to 24 hours and are then filmed and stained to see if they have yielded a growth of diphtheria bacilli. All the morphological diphtheria bacilli are retained for fermentation and virulence tests, the Hofmann's bacilli and other diphtheroids being discarded. The fermentations of the suspected organisms are established by inoculating tubes containing glucose and saccharose in Hiss' serum water. The diphtheria bacillus always ferments glucose with the production of acid but never saccharose. The virulence test, which may be done concurrently with the fermentation test, is best carried out by the intracutaneous technique. Suspensions of the organisms to be tested are made from the serum slopes and standardised by opacity to contain about 500 million organisms per c.c. Two guinea-pigs each of about 250 grm. weight are used. One of the animals serves as a control and receives at the time of the test 500 units of antitoxin intracardially, or alternatively has been given 500 units intraperitoneally on the previous day. White-haired portions of the two animals are depilated and into corresponding sites in their skins 0·2 c.c. of the suspensions to be tested are inoculated intracutaneously. The injections should be at least one-half inch apart and up to ten tests may be made on each pair of animals. The unprotected animal should be given a dose of 125 units of antitoxin intraperitoneally 6 hours after the intracutaneous inoculations have been made. The readings are usually best made on the second or third day. The virulent organism is indicated by the fact that in the unprotected animal an erythematous patch appears at the site of the inoculation about 1 to 2 cm. in diameter and progresses to superficial necrosis in 2 or 3 days. In the protected animal no reaction occurs or at most a slight red papule which disappears in a few days.

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Summary of procedure.

A brief summary of the technique outlined above may now be inserted to make clear the different stages in the detection of the virulent carrier.

The first step is the removal of the diphtheria bacillus from the nose, throat or ear by means of a swab which is at once inoculated on Loeffler's serum. On the same day that the swabs are taken, plates of Allison and Ayling's medium are poured in readiness for the next day.

On the second day the growths on the slopes are emulsified in saline and examined microscopically for diphtheria bacilli. All the growths are sub-cultured on Allison and Ayling's medium, but those in which morphological diphtheria bacilli are present are sub-cultured on an alternative medium, such as blood agar, in addition.

On the third day suspicious colonies are picked on to serum slopes and the plates of the special medium are returned to the incubator for a further 24 hours' incubation.

On the fourth day the plates are examined once more to see if any further suspicious colonies have appeared. These are transferred to serum slopes as on the previous day. The growths from the serum slopes, inoculated the previous day, are examined for morphological diphtheria bacilli and these are tested for their fermentations and virulence.

By the seventh day at latest all the diphtheria bacilli present in the original swabs should be completely identified.

The number of individuals whom one person can deal with at a time, using the foregoing technique, is about a hundred. It is well to determine that the person carrying out the operation is not himself a carrier.

Discussion of results with examples.

The technique outlined above, with a few minor modifications, has been applied by me (1931) to the detection of diphtheria carriers among Dublin school children. The investigation required the isolation of diphtheria bacilli in as large a number of instances as possible from single nose and throat swabs taken from 1000 apparently healthy school children. 2000 swabs in all were therefore submitted to examination, but of these 300 were plated on Allison and Ayling's medium only, as it was not realised at first that the medium suppresses the diphtheria bacillus in some cases. A number of strains were lost on this account before the method of plating the primary serum growths, in which diphtheria bacilli were microscopically visible, on an alternative medium was adopted. Omitting consideration of the first 300 swabs, the results achieved by the technique described above with the remaining 1700 were as follows.

In films made from the primary serum growths morphological diphtheria

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bacilli were visible in sixteen instances. They were isolated in pure culture by means of the special medium in fourteen instances, being suppressed in two cases. In these two cases they were isolated without great difficulty from blood agar plates, so that no strain was lost. Diphtheria bacilli were, however, isolated from twenty-three other primary cultures, in which they were not visible microscopically, by plating on the special medium. Assuming that the proportion of organisms suppressed by the medium remained the same for those which could not be seen in the primary cultures as for those that could, the number of organisms present, of which twenty-three were isolated, would be twenty-six. From this we may form an estimate of the efficiency of the method, the percentage efficiency being

$$\frac{(16+23)}{(16+26)} \times 100, \text{ i.e. } 93 \text{ per cent.}$$

In other words, there is a reasonable likelihood of isolating 90 per cent. of the diphtheria bacilli present in a series of swabs taken from the nose and throat. Although the numbers quoted in support of this contention are rather small, the series of swabs referred to above may be taken as representative of what is likely to be met with in practice. The number of colonies which grew on the special medium varied from only two or three up to a couple of hundred, showing that the numbers of diphtheria bacilli present in the original swabs varied from very few to quite large numbers. It should be mentioned that the above figures relate only to nose and throat swabs; the efficiency with ear swabs is liable to be less, owing to the increased difficulty of isolation caused by the frequent presence of spreading organisms.

Owing to the fact, already mentioned, that diphtheroids of various sorts are capable of producing colonies, identical in all respects with those of the diphtheria bacillus when grown on the special medium, and that they cannot be distinguished from one another microscopically in preparations from this medium, it is necessary to sub-culture more than one colony on to serum for every diphtheria bacillus isolated. The average number of colonies which must be picked in this way for each diphtheria bacillus isolated is five.

A final point of importance is that growing the diphtheria bacillus on the copper sulphate containing medium does not alter its virulence.

Applications of the method.

It will be seen from the foregoing that a technique is available which will enable one to detect the vast majority of carriers by examination of material removed from suspects at a single swabbing.

The detection includes determination of virulence which is, of course, all-important from the preventive point of view. The application of the method to the detection of carriers in the general population is indicated by me elsewhere (1931). The application of the method in a few other specific instances

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may now be considered briefly. The cases it is proposed to deal with presume sporadic outbreaks of diphtheria in the following institutions:

(*a*) a day school;
(*b*) a small residential institution;
(*c*) a large residential institution;
(*d*) a general hospital.

To consider first the case of the day school. If it is a small school of young and consequently susceptible children, all should be swabbed and dealt with in accordance with the technique described above. The children may be Schick tested by an assistant at the time that the swabs are taken, in order to find the susceptibles preparatory to immunisation. As each child is finished he should be sent home and the school may be closed for four days until the isolation of the morphological diphtheria bacilli in pure culture is complete. The school may then be re-opened, those children who have been found morphological carriers being kept at home. These in due course are separated into virulent and non-virulent carriers, the latter being allowed to return to school, the former being retained and treated. It is advisable to swab the members of the families of virulent carriers in order to determine whether or not there are other carriers in the home.

If the school is too large for all the children to be swabbed, only those classes which have been subject to outbreaks of diphtheria may be dealt with or else the Schick test may be performed first to distinguish susceptibles from non-susceptibles, the latter being then swabbed as the most likely to harbour virulent diphtheria bacilli. Schick positive individuals may, however, also harbour virulent diphtheria bacilli and these have actually been isolated from them as shown by McGuire and Hitchens (1923) and Beattie and Herron (1926). Considering that 80 per cent. or more of adults are Schick negative in certain communities and that their immunity is due to contact with virulent diphtheria bacilli, without the occurrence of the disease in most cases, it is probable that many Schick positive individuals at one time or another are carriers of the diphtheria bacillus in their noses or throats. As to the duration of the carrier state or the numbers of diphtheria bacilli carried, in such instances, there is no information, and this aspect of the subject would seem to lend itself to much needed study by means of the technique described in this paper. The three Schick positive carriers detected by McGuire and Hitchens, among seven virulent carriers tested, apparently harboured considerable numbers of the organisms.

The proposition of Okell, Eagleton and O'Brien (1924), that Schick positive reactors never harbour virulent diphtheria bacilli, detectable by ordinary swab-culture methods, unless they are suffering from or are incubating diphtheria would seem to require further proof.

In dealing with a small residential institution, it is well to swab all the residents, but in large residential institutions, it may only be possible to swab the Schick negative reactors. If the cases of diphtheria, however, have

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occurred in only one section of a large residential institution divided up into sections, then the best procedure would be to swab all the residents in that section. It should be noted that in dealing with residential institutions, isolation is desirable as soon as possible. In consequence those carriers who give a growth of morphological diphtheria bacilli, as judged by examination of films from the primary serum cultures, should be isolated at once, the others being isolated subsequently as their detection is completed.

Sporadic outbreaks of diphtheria in a general hospital are a fairly common cause of great inconvenience. The carriers are to be sought among the members of the staff of the hospital and the occupants of the wards in which the cases have occurred. It is well to remember in dealing with this type of outbreak, that the diphtheria bacillus sometimes infects wounds or similar lesions and may have to be sought in some such situation. As the numbers of individuals to be dealt with in this type of outbreak are not very large, as a rule, the method should be very readily applicable and should ensure an immediate arrest of an outbreak of diphtheria. As in the case of a residential institution, isolation of those carriers, who give a positive culture of morphological diphtheria bacilli in the primary serum growth, should be carried out at once, isolation of the others following in due course.

Summary.

The technique of detecting diphtheria bacillus carriers has found little recognition in the literature of diphtheria.

A method of detecting diphtheria carriers by single swabbings is described.

The method is somewhat elaborate but is highly efficient.

The results which may be obtained with it are discussed.

Its application in certain specific cases is briefly considered.

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