AN INVESTIGATION INTO THE CARRIER CONDITION AS IT OCCURS IN DIPHTHERIA.

By

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Being a Thesis for the Degree of Doctor of Medicine, University of Glasgow.

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FOREWORD.

At the suggestion of Dr.F.H.Thomson, Medical Superintendent of the North-Eastern Hospital, the Metropolitan Asylums Board decided to undertake an investigation into diphtheria carriers. A scheme of laboratory investigation was therefore drawn up in June, 1925, by Dr.J.E. McCartney, Director of the Board's Research and Pathological Services, and approved by the Scientific Advisory Committee*. It was decided to employ a whole-time bacteriologist to carry out the proposed scheme, and I was fortunate enough to secure the position. A laboratory was equipped at the North-Eastern Hospital, and work on the investigation commenced in October, 1925.

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The following thesis embodies the work carried out during the investigation. This work has continuously been under the supervision of Dr.J.E. McCartney, Director of the Board's Research and Pathological Services, and the results and conclusions have been approved throughout by the Scientific Advisory Committee.

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I N T R O D U C T I O N.

INTRODUCTORY.

The problem of the carrier condition in infectious diseases has been one of paramount and increasing importance in medicine for many years. Even before the organismal origin of disease was thought of, the idea had been expressed that apparently healthy persons could communicate a disease to others who had been in contact with them. Later, towards the end of last century, came the discovery of the causative organisms of so many diseases, and with that the revolutionary change in our ideas of infectivity and epidemiology. One of the earliest and most interesting results of succeeding investigation was the definite proof that persons, apparently well and especially when recovering or recovered from acute infectious diseases, still harboured the causal agent and were able to communicate it to others.

The earliest reported work on this subject was done by Koch and his co-workers on typhoid, from 1902 till 1905, in South-West Germany, although Houston-Smith drew attention to the same subject in the Goulstonian lectures of 1900.

Since that time a great deal of work has been done on the carrier problem and much has been written on the subject.

Many diseases, especially diphtheria, cholera, dysentery and cerebro-spinal meningitis have been shown to give rise to a carrier condition, and within recent years it has been suggested that more diseases may be transmitted in a similar manner.

In spite of this work, however, our knowledge of the basic principles underlying the carrier condition are still far from satisfactory, and in most cases our treatment of the condition has been equally unfortunate.

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HISTORICAL.

Trousseau[1] in 1868 noted and published the fact that severe cases of diphtheria could apparently transmit the disease in a mild form to others. These, in their turn, could again transmit the disease to yet a third group of contacts. The most interesting feature of this statement is that at the time of its publication the aetiological agent of diphtheria had not been discovered. Bristowe[2] in 1884 recognised on clinical grounds that persons who had recovered from diphtheria for a period of two or three weeks could still communicate the disease. Loeffler[3] in the first description of his work on the bacillus diphtheriae noted the fact that one of the virulent strains which he isolated had come from the throat of an apparently healthy person. Later, when the bacillus diphtheriae had been generally accepted as the causative organism in diphtheria, evidence accumulated as to the definite existence of a carrier state, and the importance of this phase of the disease. Thus, Roux and Yersin[4], in 1890, discovered the bacillus diphtheriae in the throat of a patient two weeks after the membrane had disappeared; while Loeffler[5] in the same year also recovered the organism from a throat three weeks after the patient's temperature had returned to normal. This fact was confirmed by Abel[6], in 1894, Morse[7], 1894, Schäfer[8], 1895, and Sévestre and Méry[9], in 1895.

About this time interest in the carrier problem waned somewhat owing to the promulgation of two new theories. These were, firstly, the soil theory of Pettenkofer, who suggested that the soil was the greatest reservoir of infection; and, secondly, the statement by von Behring[10], in 1901, that measures for the isolation of carriers, etc., were quite useless owing to the ubiquity of the diphtheria bacillus. In the light of further investigation, however, these theories have been discredited, and the importance of the diphtheria carrier as a prime factor in the transmission of the disease has again been emphasised.

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DEFINITION AND CLASSIFICATION OF CARRIERS.

Before dealing further with the question of diphtheria carriers, it is necessary to define and classify carriers as they occur in this disease.

DEFINITION.

A carrier is a person who harbours and is capable of transmitting the organism or virus of a disease, without showing clinical symptoms of that disease.

The organisms concerned in diphtheria is the bacillus (corynebacterium) diphtheriae of Klebs and Loeffler, hereafter usually referred to throughout the text as the Klebs-Loeffler bacillus, or K.L.B.

CLASSIFICATION.

The question of classification is much more difficult. The issue has been confused by the lack of distinction existing in the literature between true carriers and persons incubating or suffering from a chronic form of any disease which is liable to give rise to a carrier condition. Special difficulty has arisen in dealing with diphtheria carriers from the known existence of virulent and non-virulent strains of the Klebs-Loeffler bacillus, as well as several varieties of diphtheroids, notably the bacillus of Hofmann. In the

absence of any generally accepted proof that diphtheria bacilli and members of the diphtheroid group are the same organisms in different stages of a life cycle, or living under altered conditions, we must assume that such is not the case.

The only really important carrier, therefore, comes to be that one who harbours TRUE, VIRULENT DIPHTHERIA BACILLI, and to this type we will confine our attention.

The classification of the Klebs-Loeffler bacillus itself is of such great importance that the scheme which has been adopted will be given here in some detail, as this must form the foundation upon which any work on diphtheria carriers should rest.

The scheme upon which I have based the identification of the B.diphtheriae is taken from the classification introduced by Park and Beebe[11] in their important work of 1894, and later amplified by Graham Smith[12] in 1908. The basis of this classification is the fermentative reactions of the organisms to glucose and saccharose, and the result of the virulence test. Three main divisions of organisms morphologically resembling K.L.B. are recognised, as follows:-

(1). Those bacilli which ferment neither glucose nor saccharose and are non-virulent for a guinea-pig - <u>Hofmann's Bacillus.</u>

(2). Those bacilli which ferment both glucose and saccharose with production of acid - saccharose fermenters, e.g. Bacillus Xerosis, - also non-virulent.

(3). Those bacilli which ferment glucose with production of acid, but which do not ferment saccharose. This type is further sub-divided into:-

(a). Those bacilli which are virulent for a guinea-pig - Virulent diphtheria bacillus or true Klebs-Loeffler bacillus.

(b). Those bacilli which are non-virulent for a guinea-pig, - non-virulent or avirulent diphtheria bacillus - bacillus maculatus of Graham Smith, bacillus coryzae segmentosus.

Carriers under the present investigation were entirely confined to those who harboured organisms belonging to the group 3a. In other words, before any persons could be considered carriers, they had to yield an organism morphologically resembling K.L.B., which in pure culture produced acid from glucose, but not from saccharose, and which was virulent for a guinea-pig. Throughout the investigation this classification was adhered to rigidly.

The methods of isolation, and the testing of the virulence of the organisms will be fully described later.

The actual classification of carriers must now be considered. Ledingham and Arkwright[13] in their work on, "The Carrier Problem in Infectious Diseases" give a very serviceable classification of diphtheria carriers. These authors divide carriers into three main groups, as follows:-

CLASS I. Convalescent Carriers.

This group includes all those who have had diphtheria and in whom, after an apparent return to health with disappearance of local lesions, the diphtheria bacilli still persist.

This class is further sub-divided into:- (a). those who are apparently in perfect health at the time of examination, and (b). those who are suffering from some pathological condition, however slight. This distinction, unfortunately, is very difficult to maintain in practice, as it is not easy to place accurately those cases with enlarged tonsils and adenoids, or slight catarrhal conditions.

CLASS II. This class is made up of those cases in which no attack of clinical diphtheria has been recognised. It includes the so-called "bacteriological diphtheria" cases. This group corresponds to the "Bazillenträger" of the German authors, or,

as they are sometimes termed, "healthy carriers".

CLASS III. Chronic Carriers.

This group has been arbitrarily fashioned to include those cases which have harboured diphtheria bacilli for at least twelve weeks. For our investigation, we found it advisable to adopt this period, and to label a case a "chronic carrier" when diphtheria bacilli had persisted for twelve weeks. This period was chosen as it proved to be the best practicable basis upon which to work. Thus the case was not detained too long in hospital before officially becoming a carrier, while any doubt as to the transient nature of the carrier state was safely eliminated.

A fourth group may be added. This comprises the so-called "Incubationary Carriers". These, however, are really not carriers in the proper sense of the word, but are merely persons incubating the diphtheria bacillus before the clinical symptoms of the disease have made their appearance. The Medical Research Council in its report on Diphtheria[14], 1923, points out that such cases can be sharply defined from true carriers by the result of the Schick test. In true carriers

the result of the Schick test should be negative, whereas in the so-called "Incubationary Carrier" the result is positive.

An extreme example of this type is the "Precocious Carrier". The person concerned here may harbour K.L.B. for quite a long period before any clinical signs of the disease appear. Wesbrook(15), in 1898, noted a case in which bacilli were present in the throat for two weeks before the onset of the clinical attack of diphtheria.

True carriers must also be differentiated from persons who, though suffering from a mild attack of diphtheria, show few signs or symptoms of the disease. There can be no sharply defined line drawn, however, between these two types of cases, as the division is purely artificial. The only essential difference rests in the variation found in the toxin-antitoxin balance of the blood.

For the purposes of this investigation the only carrier whom we will consider is that one whom we have termed the "Chronic Carrier", namely, the person who has harboured true K.L.B., as already defined, for a period of at least twelve weeks. By confining ourselves to this group we can be reasonably certain that we are dealing with true and not with spurious carriers, and our investigation and results will thus rest upon a much more certain foundation.

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PREVIOUS WORK.

A very extensive literature has been built up around the subject of diphtheria carriers.

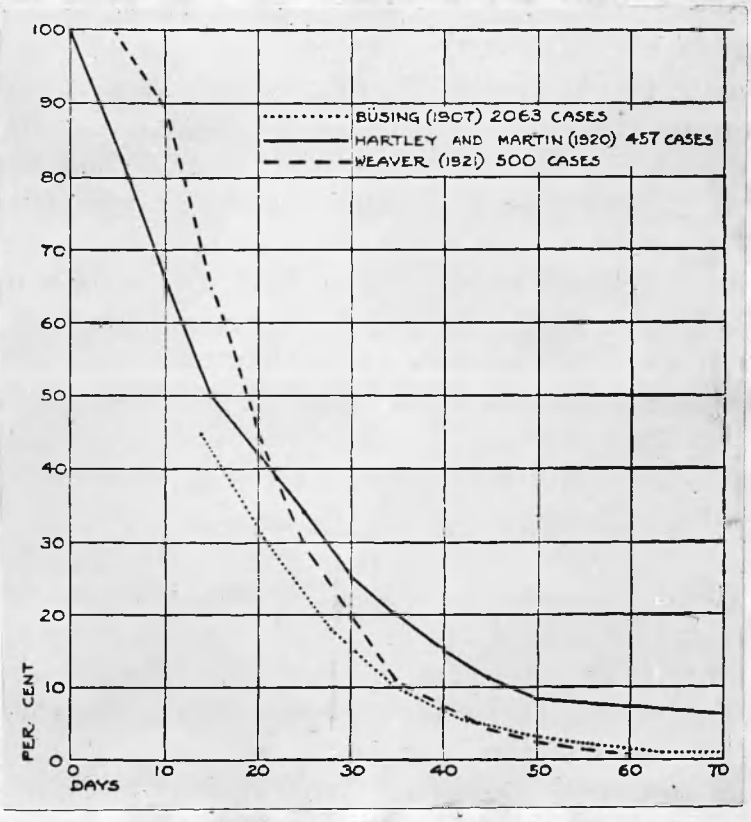
As has already been stated the recognition of the carrier condition in diphtheria was reported as far back as 1884. The first important work upon the subject was done by Park and Beebe[11], in 1894, and this work was so well done and so comprehensive in its scope that their results still form the basis of most of the modern work on the subject. The main result of their investigations was to prove that bacilli normally tend to disappear from the throat and nose during convalescence, but that in some cases this disappearance is delayed, although in all cases ultimate disappearance is the rule.

Statistics have accumulated as to the rate of disappearance of the bacilli during convalescence, and some of these are of the utmost importance. Hartley and Martin[16], in 1919-20, did excellent work on this subject, and their results are generally accepted (within limits to be presently mentioned) as being an accurate representation of the usual sequence of events. These workers found that the rate at which convalescent carriers ceased to carry was a direct function of the number of positive carriers at any time, and that,

consequently, the disappearance of bacilli from the throats followed a simple logarithmic law. They showed that five per cent of the surviving positive convalescents became negative every day. The actual work done was of an almost ideal character, but two criticisms can be made as to the value of their deductions. In the first place, the work was done on adult males only, and as the great majority of chronic carriers are children, this fact must necessarily detract considerably from the value of their results. Secondly, no distinction was drawn between carriers who harboured bacilli in the throat and those who harboured bacilli in the nose, and, as will be shown later, this point is one of great importance in the study of chronic diphtheria carriers.

More work on this subject was done by Büsing[17], 1907, and Weaver[18], 1921. The latter, dealing mainly with children, found that nine per cent became negative daily. As will be seen, there is considerable discrepancy between his results and those of Hartley and Martin referred to above. The fact that Weaver took his cultures at intervals of one to three days, whereas Hartley and Martin took their cultures at intervals of seven days only, as well as the variation in the ages of the carriers concerned, probably explains this difference in results. The following

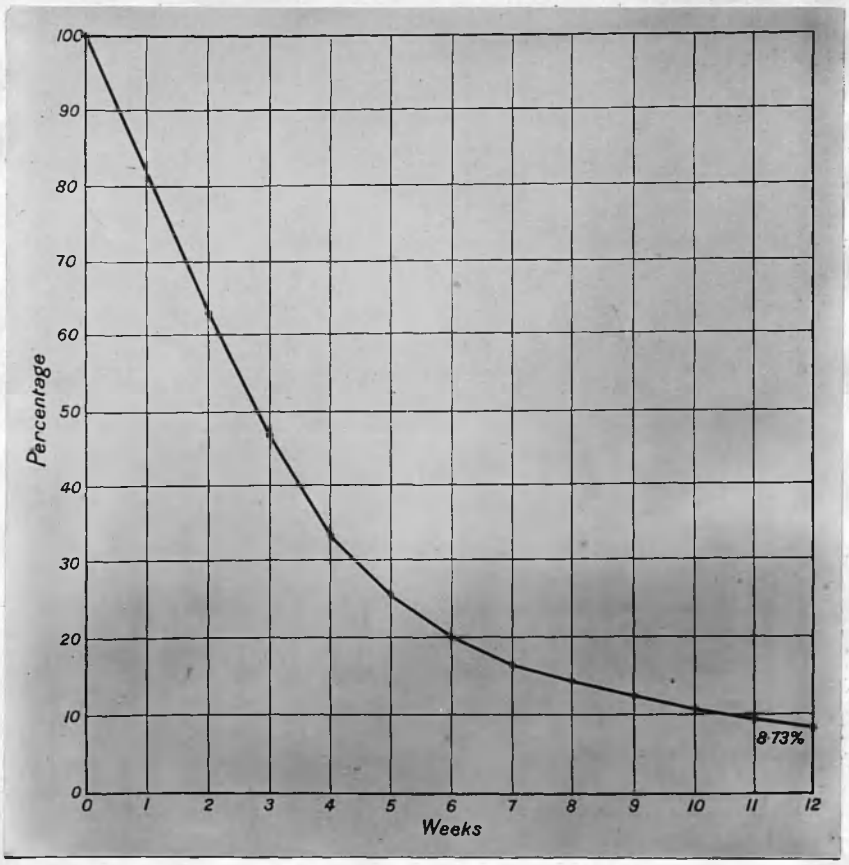
is a graph taken from the Medical Research Council's Report on Diphtheria, page 335, giving a comparison between the results of Büsing, Hartley and Martin, and Weaver, regarding the disappearance of K.L.B. at different stages during convalescence:-



The question of the disappearance of K.L.B. from the nose and throat of patients during convalescence from diphtheria has recently been further investigated most carefully at the North-Eastern Hospital, Metropolitan Asylums Board, by Thomson, Mann and McCartney[19]. Their work is particularly applicable here, as the present investigation was

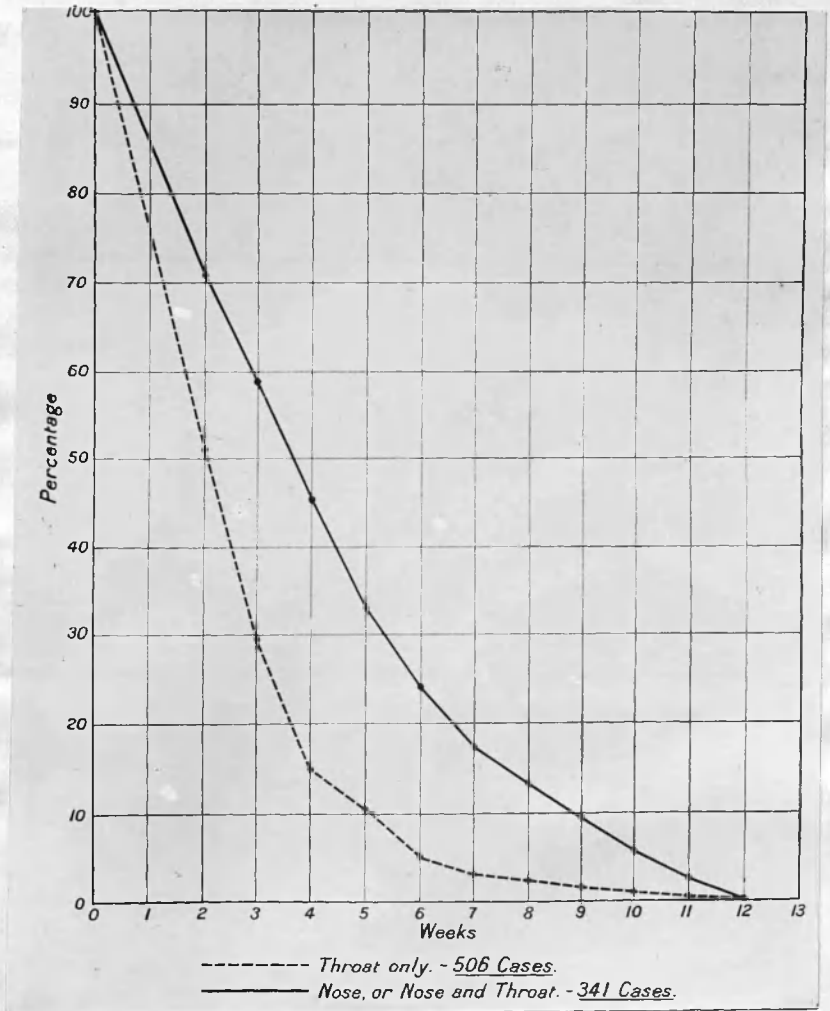
carried out in the same hospital, and several cases used to make up these authors' total were also used in the investigation forming the basis of this thesis. These workers, moreover, stress the great importance of the investigator himself taking the cultures, and, in addition, draw a sharp line of distinction between the statistics dealing with pure throat cases and those dealing with cases where the nose only or the nose and throat were involved. Their results, therefore, will be given in some detail.

The following is a graph which they publish showing the rate of disappearance of the bacilli in positive cases each week, from the second to the twelfth week, when the case officially became a carrier:-



It will be seen that 8.73 per cent of the total cases became carriers at the end of 12 weeks.

They publish also a very instructive chart showing the comparative rate of disappearance of diphtheria bacilli in (a). cases in which the organisms were present only in the throat, and (b). cases in which organisms were present only in the nose, or in the nose and throat.



From this it will be seen that cases in which the nose was involved took longer to become negative than cases in which the throat only was involved. Their figures in more detail are as follows:-

Age Group	Percentage of negatives in first eight weeks.	
	Throat only affected.	Nose and Throat affected.
Up to 7 years	97.2 per cent	83.6 per cent
7 to 13 "	98 " "	90.4 " "
Over 13 "	100 " "	97.7 " "

These figures are of great importance and this point seems to have received insufficient attention in the past.

These authors also stress the fact that a prolonged negative interval may ensue in a positive carrier, this negative period extending up to 8 weeks.

In a series of 104 carriers the following are their observations and figures:-

"To show how fallacious may be the apparent disappearance of the bacilli from the throat or nose, on 115 occasions these 104 carriers showed considerable intervals during which bacilli could not be recovered, but in all the bacilli reappeared.

The intervals were as follows:-

On 1 occasion the interval was 8 weeks during which the bacilli could not be found.
" 4 occasions " " " 6 " " " " " "
" 9 " " " " 5 " " " " " "
" 24 " " " " 4 " " " " " "
" 31 " " " " 3 " " " " " "
" 46 " " " " 2 " " " " " ". "

In addition they publish a graph (shown below), giving the age incidence of the 104 carriers examined.

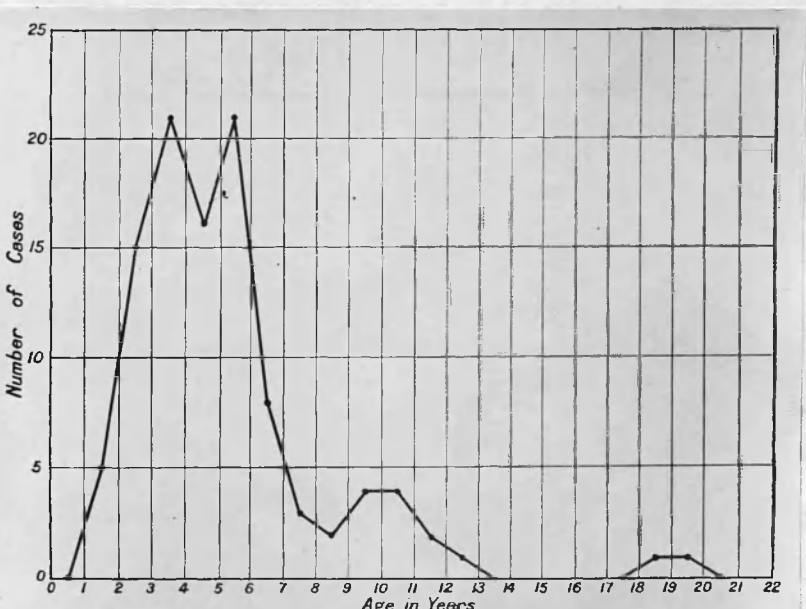


FIG. 3.—Chart showing the age incidence of diphtheria carriers.

Of these 104 carriers, the bacilli were limited to the throat in 29 cases, were found in both the throat and nose, or in the nose only in 69, in the nose and ear in 4, and in the ear only in 2.

Of these 104 carriers, the bacilli were limited to the throat in 29 cases, were found in both the throat and nose, or in the nose only, in 69; in the nose and ear in 4; and in the ear only in 2.

They add that "Diphtheria bacilli normally tend to disappear during convalescence, and the problem of the

diphtheria carrier is to ascertain why the bacilli persist. This persistence indicates a failure of the natural process of elimination, and if the factor or factors preventing the normal disappearance of diphtheria bacilli could be ascertained, the carrier problem would be simplified".

Various cases are recorded in the literature of carriers who remained positive for unusually long periods. Thus, Abel[6] in 1894, reported a case in which the bacilli persisted for 65 days. McGregor[20] in 1898, also reported a case in which the bacilli persisted for 6 months, while Schäfer[8], in 1895, reported a case in which the bacilli persisted for over 7 months.

Much work has been done on the possible transmutation of the various strains of diphtheroids, especially the bacillus of Hofmann, into virulent diphtheria bacilli. The theory that this transmutation might occur was advanced by Sacquépée[21] and others in France, in 1910, but no substantial proof of this has been forthcoming, although Hewlett and Knight[22], 1897, and Salter[23], 1899, did claim to have succeeded in actually bringing about this change. On the other hand, Clark[24], in 1910, made extensive attempts to change the character of the bacillus of Hofmann, but had no success; and though Goodman[25], in 1908, claimed to have increased the power of Hofmann's bacillus to ferment glucose, most workers have failed signally

to change in any way the non-virulent diphtheroid into a virulent diphtheria bacillus, or, conversely, to decrease the virulence of a diphtheria bacillus to that of a Hofmann strain. Graham Smith[8], in 1908, collected the records giving particulars of 10,570 healthy persons, and of these 18.2 per cent yielded Hofmann's bacilli in the throat. Cobbett, as reported by Graham Smith[26], in 1904, found that 39 per cent of 1,724 children who had been in contact with diphtheria cases were harbouring the bacillus of Hofmann in the throat.

Roux and Yersin[4], 1890, and Funck[27], 1894, found that the virulence of a strain of the diphtheria bacillus could be exalted for a guinea-pig, when injected along with streptococci. This phenomenon, however, only occurred when virulent strains were used, and did not appear when the bacilli were completely non-virulent.

Moss, Guthrie and Marshall[28], in 1921, established a carrier state in man, using non-virulent diphtheria bacilli. They noted that these bacilli remained non-virulent throughout the period of carrying. There does not appear to have been any further work done on this subject.

The occasional occurrence of the non-virulent variety of the diphtheria bacillus has received considerable attention from time to time in the literature. Morphologically, and

from the sugar tests, it is quite indistinguishable from the true virulent Klebs-Loeffler bacillus, and, unless a virulence test is performed, patients may be detained for prolonged periods in hospital when harbouring only a non-virulent organism. This fact is obviously sufficient to warrant much further investigation upon the subject, and the establishment, once and for all, of the true relationship between the two varieties. Cases have been noted where virulent and non-virulent strains of the diphtheria bacillus have been isolated simultaneously, or at intervening short intervals, from the same patient. The present investigation has furnished several such instances. Okell and Parish[29] state that out of 626 positive cultures, taken from cases of diphtheria, two instances were observed where virulent and non-virulent cultures were isolated from the same patient. The same authors examined cultures from 758 carriers and obtained three cases where they were able to isolate virulent and non-virulent bacilli from the same carrier. They also obtained four cases in which a virulent diphtheria bacillus was replaced, on further swabbing within a few weeks, by a non-virulent strain. They did not, however, obtain a single case in which a non-virulent strain was later replaced by a virulent strain. It would appear to be necessary to test the virulence of a large number of strains from the same case, and

also to use a large series of cases, before any definite opinion could be passed on this subject.

The position seems, therefore, to be that, though several observers have claimed to have been able to transpose one variety of diphtheroid into another variety of diphtheria bacillus, this fact has never received proper substantiation. As far as we know, then, each variety of diphtheroid and diphtheria bacillus is a separate and distinct entity and remains so throughout its life history. Acting on this assumption it is the custom, at present, to ignore all cases except those harbouring true virulent diphtheria bacilli. The fact that no "return cases" have been reported when carriers of non-virulent bacilli have been discharged from hospital, supports, though it does not entirely confirm this opinion.

Statistics have been produced to show the percentage of healthy non-contacts and also of healthy diphtheria contacts who, on investigation, proved to be carriers. The following may be cited as typical examples:-
Peck[32], in 1895, found that 31 per cent of 100 boarders in a school were carriers. Kober[31], in 1899, examined 128 diphtheria contacts, and found that 15 (11 per cent) were positive. Of these, 5 had had "sore throats", however, and were probably mild cases of diphtheria. Asser[30], in

1903, examined 89 healthy men in a cavalry regiment, and found that 17 (19 per cent) were carriers. In addition, Schelle[33], in 1906, gave the history of a school teacher's family in which one young female, aged 7 years, infected six out of the remaining 7 members, all of whom became carriers. These figures show the high incidence of carriers among the general population, both contact and non-contact, and emphasize the great potential danger which they constitute to their associates.

Many theories have been advanced as to the origin of the carrier state and, based on those theories, many methods have been employed to rid carriers of their infecting bacilli.

The commonest explanation of the carrier state was, and still is, that the bacilli persist in a chronic carrier because of the existence of some local pathological condition. The presence of this abnormality is supposed to provide the bacilli with a suitable nidus, in which they can multiply with ease and rapidity. Such conditions might be found in enlarged or diseased tonsils and adenoids, or in infected sinuses.

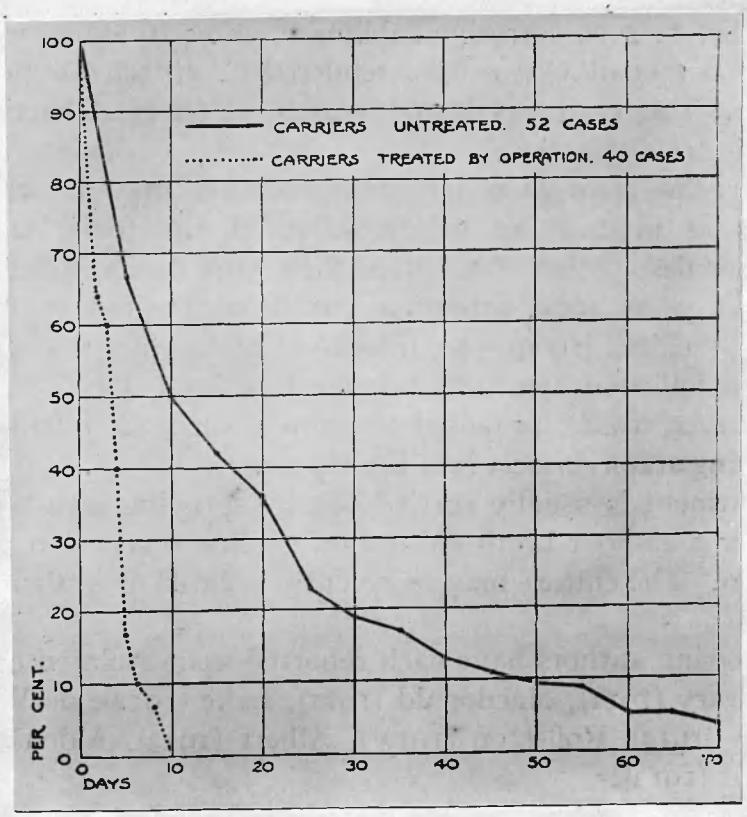
The following figures show the importance of the tonsil in the maintenance and spread of the carrier condition in diphtheria. Koplik[34], in 1894, found diphtheria bacilli deep in the crypts of apparently healthy tonsils. Brown[35], in 1916, discovered diphtheria bacilli in microscopical

sections from the tonsils of 7 carriers. The work of Kretschner[36], in 1911, and Albert[37], in 1913, was of still greater importance. They showed that even when cultures from the tonsillar surface were negative, K.L.B. could be recovered from the crypts by squeezing out the material. The importance of this aspect was further brought out in an investigation by Dudley[38], in 1923, who showed that the complete absence of chronic carriers in a heavily infected school was probably due to the fact that the boys in the school, being candidates for the Royal Navy, were all subjected to a careful medical supervision. This meant that all local conditions in the throat were immediately remedied as they arose.

Treatment by the removal of tonsils has been practised by various workers with more or less success. Pegler[39], in 1905, was the first to suggest carrying out this form of treatment. Rabinoff[40], in 1916, Miller, Ruh and Perkins[41], in 1916, and Keefer, Friedberg and Aronson[42], in 1918, also reported rapid cures by tonsillectomy. It has been the custom for some time at the North-Eastern Hospital to remove the tonsils of suitable carriers, and the results there have been very successful.

The following graph, taken from the Medical Research Council's Report on Diphtheria, already quoted (p.368) shows

the comparison between the rate of disappearance of the bacilli in untreated cases and in cases treated by tonsillectomy, as obtained by Weaver[18], in 1921. The percentage of negatives per day in the treated cases was 35 as compared with 4 in the untreated cases.



The literature on the question of tonsillectomy is not extensive, but there is enough evidence to show that treatment by this method in selected cases is usually efficacious in ridding the patient of the carrier condition. No stress seems to have been laid on the influence of a positive nose culture

upon the effect of treatment by tonsillectomy, yet this point is extremely important. Special attention was paid to this in the present investigation, and the results obtained will be given later.

There are no important references in the literature to the effect of sinus infection upon the carrier condition in diphtheria.

Rabinoff[40], in 1916, and Weaver[18], in 1921, report immediate cures after the removal of foreign bodies, such as boot buttons and cherry stones, from the nose.

Treatment by local antiseptics, such as camphor, iodine oil, alcohol, chlorine, menthol, thymol, pyocyanase, iodex, etc., has been almost uniformly unsuccessful. Any beneficial results obtained were found to be only temporary.

Loeffler[3], in 1895, tried a mixture of alcohol, toluol and liquor ferri perchloridis in the local treatment of diphtheria, and found it quite successful. He used this same mixture in the treatment of artificially produced wounds in guinea-pigs with equally good results.

More recently Filer[43], in 1920, protected artificially injected guinea-pigs by the use of trypaflavin. Reinhardt[44], in 1922, confirmed these results in vitro. He found that 1/1000 trypaflavin was sufficient to render diphtheria bacilli

non-infective in two minutes. This work, however, has not been applied to carriers.

Treatment by toxin and toxin-antitoxin mixtures has been tried on various occasions, but has not proved successful. Wassermann[45], in 1902, and L. Martin[46], in 1903, claimed good results from treatment in the form of pastilles made from dried anti-bacterial serum. In a series of 72 cases, Dopter[47], in 1905, claimed considerable success on using this powder by the method of insufflation. Kretschner[36], in 1911, had no success with bacterial serum, and Roskam[48], in 1918, considered the benefits of Martin's anti-bacterial serum to be non-specific, as anti-meningeal serum would produce equally good results. Bauer[49], in 1914, used a toxin-antitoxin mixture, but his results do not cover enough ground to be conclusive. This lack of success is natural, as the blood of carriers must necessarily contain a high antitoxin content.

Bowen[50], following the lines suggested by Besredka[51], did some work in 1925 on the production of local immunity against diphtheria by the use of minute harmless doses of toxin. He claims to have succeeded in protecting the injured conjunctival surface of a guinea-pig in this way from the effect produced by the local application of a suspension of virulent K.L.B. Very little other work has been done on this subject with the exception of that done by Arloing[52], in 1901, who considered

that the nasal mucus had a protective influence against diphtheria. This work, however, has never been confirmed.

Vaccines have been employed in the treatment of the carrier condition with varying results. Petruschky[53] appears to have originated the idea in 1908. He used dead virulent diphtheria bacilli, and 5 of his 6 cases ceased to carry during treatment. His results, however, are not conclusive. Walker Hall and Scott Williamson[54], in 1911, have reported 6 cases treated with vaccines. They claim 4 "cures" after four to five weekly injections of their prepared vaccines. Of later years, Brownlie[55], in 1920, (50 cases), and Fraser and Duncan[56], in 1920, have used vaccines; while Hewlett and Nankivell[57], in 1912, used endotoxin in the attempted cure of the carrier condition. The same criticism applies to all these results, namely, that they are too isolated to form the basis of any definite opinion. These vaccines were, in every case, made from the Klebs-Loeffler bacillus and given with the idea of attacking the bacillus directly,in the region affected. There does not appear to be any reference in the liberature to vaccines having been given with the idea of altering the flora of the nose and throat. In other words, no one appears to have attempted the treatment of a carrier by means of a vaccine composed of the organisms associated with the Klebs-Loeffler bacillus in the

affected area. Stévenin[58], in 1917, and Weaver[18], in 1921, however, drew attention to the fact that an attack of follicular tonsillitis in a carrier freed the carrier from his infection. Schiøtz[59], in 1919, suggested that this observation might be applied practically, by spraying the throat with living staphylococci. In other words, he proposed to induce an attack of tonsillitis. Reports on a few cases treated in this manner have been made by Page[60] and Macdonald[61], in 1911, and by Rolleston[62] and F.L. Wright[63], in 1913. Hewlett and Nankivell[57], in 1912, Wood[64], in 1913, and Ruh, Miller and Perkins[41], in 1916, used organisms other than staphylococcus aureus to bring about the same result. Thus Wood used a lactic acid bacillus spray. The number of cases treated, however, was meagre, and the results isolated.

The occurrence of diphtheria bacilli in the lower animals does not appear to have much significance in causing an attack of diphtheria, or in establishing a carrier condition in man. The literature on this subject is quite extensive, but most of it deals only with isolated cases, and so is not of much importance. K.L.B. have been isolated by Cobbett[65], in 1900, from a horse with nasal discharge; by Brandt[66], in 1908, from a dog; by Ashby[67], in 1906, and Henry[68], in 1920, from the udders of a cow; and by Simmons[69] from a cat. In the last mentioned case, however, the bacilli which were

isolated fermented saccharose, and so some doubt must be thrown on the significance of the finding. Diphtheria bacilli may also persist for considerable periods on inaminate objects, such as pencils, ink-wells, soiled handkerchiefs,etc. H. Cristiani[70], in 1906, claims to have isolated diphtheria bacilli in considerable numbers from pencils which had been sucked by carriers, and then put away in a box for fifteen days. In addition, he found Klebs-Loeffler bacillus in an inkstand forty-two days after the apparent date of infection. He did not do fermentation or virulence tests, however, and his results, therefore, lose much, if not all, of their significance. Seligman[71], in 1911, however, obtained virulent Klebs-Loeffler bacilli from a crack between the floor boards of a room in which an early case of diphtheria had vomited "some time previously".

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SUMMARY OF PREVIOUS WORK.

It will be seen from the selected data given that a very considerable literature has arisen around the subject of diphtheria carriers.

The work has mainly been carried out along two lines. In the first place, a good deal of work has been done upon the rate of disappearance of bacilli from the throats of infected patients, and also upon the incidence of the carrier condition in contact and non-contact members of the population. In the second place, investigation has been much concerned with the means of ridding established carriers of their infective condition.

The treatment of carriers has been generally that of a frontal attack upon the diphtheria bacillus. Little work has been done in estimating the influence exerted upon the carrier condition by the organisms present along with the K.L.B., together with the possible variation in the viability of the K.L.B. thereby produced.

Another criticism may also be levelled against the previous work done on diphtheria carriers. Some observers have not been too particular in stating whether or not the organisms which they isolated were typical, virulent diphtheria bacilli. Any piece of work which ignores this vital point must, as already

stated, lose a great deal of its significance.

In addition, many conclusions arrived at by various workers have been based upon results obtained from the investigation of only a few isolated cases. The carrier problem, however, is too widespread and too important to warrant this procedure, which should be remedied in all work done upon the subject.

There appears, therefore, to be scope for the investigation of diphtheria carriers along several well defined lines. In the first place, much of the work previously done is of such importance that it would admit of further investigation for the confirmation or otherwise of the results obtained. Secondly, there still remain some important points which have received inadequate attention in the past, or, in some cases, have been entirely neglected. These will be more fully discussed when the problem comes to be stated.

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THE IMPORTANCE OF DIPHTHERIA CARRIERS.

It has been shown from the published records of various workers (Prip[72], 1901, Cobbett[73], 1901, Cuno[74], 1902, Seligman[71], 1911, Forbes and Newsholme[75], 1912, and others), that the existence of diphtheria carriers is of great importance to the community. They have indeed been proved to constitute a grave potential danger to their associates, more especially if these are young children.

There are three main aspects from which we may judge the importance of diphtheria carriers. These are as follows:-

(1). As to their medical importance.

(2). As to their social importance.

(3). As to their economic importance.

(1). MEDICAL IMPORTANCE. The importance of diphtheria carriers from a medical standpoint is obvious. The carrier condition constitutes a variation from the normal and, as such, calls for adequate medical treatment. In addition, the carrier state has been shown to be in many cases the result of some pathological condition existing in the nose, throat or ear, etc. This underlying condition, whether it be enlarged tonsils, catarrh of the nose, antrum disease or otorrhoea will also require to be investigated and treated in the requisite manner. The various forms of treatment already noted as having been

carried out in the attempt to rid carriers of the bacilli which they harbour, will be ample evidence of the large amount of work already done from a purely medical or surgical standpoint with regard to this condition.

(2). SOCIAL IMPORTANCE. The social or sociological importance of diphtheria carriers cannot be over-estimated. It is becoming increasingly evident that the epidemiology of many infectious diseases including diphtheria is very greatly influenced by the existence among the general population of unsuspected healthy carriers of these diseases. It is very difficult to state exactly how many cases of diphtheria can be traced to any one particular carrier, as so many outside factors intervene to confuse the issue. For instance, it is usually very difficult, if not impossible, to ascertain how many persons any one particular carrier has been in contact with, and so the number of "return cases" due to any carrier or group of carriers cannot be estimated. This is also the view held by Ledingham and Arkwright who state in their book already quoted[13] (p.214), that, "A single case will often apparently effect the invasion of large numbers of persons, but it is difficult to be certain how many are infected directly". Opinions, however, differ greatly on this subject. Thus, Seligman[71], basing his results on several carefully

investigated outbreaks of diphtheria, states that a carrier may remain in a community of children for a prolonged period without giving rise to a single case of diphtheria. Schelle[33], on the other hand, maintains that practically every person who comes into close contact with a case of diphtheria sooner or later becomes a carrier. In addition, many outbreaks of diphtheria, especially in institutions, hospitals, schools, etc., have been apparently traced to the presence, among the inmates, of a carrier of diphtheria (see authors quoted at commencement of this section). This was confirmed in the present investigation, as several nurses who were admitted to the North-Eastern Hospital as carriers had already given rise to small outbreaks of diphtheria in the hospitals to which they had been attached. Another similar instance occurred during the present investigation when an outbreak of diphtheria among the boys of the Training Ship "Exmouth" was found, on examination of swabs taken from the entire personnel of the ship, to be due to the presence of several carriers. Several of these carriers were later incorporated in the number of cases forming the material upon which I have based this thesis.

The importance of the age and sex of the carrier has occasionally attracted attention as to the relative frequency with which persons in the different age periods and of

different sexes become carriers. Thomas[76], in 1904, stated that carriers were two or three times more numerous among males than among females. This has not been borne out in the present investigation, as the sex incidence of the carriers dealt with has been practically equal.

(3). ECONOMIC IMPORTANCE. The economic importance of diphtheria carriers is apt to be forgotten when considering the significance of the carrier in the life of the community. The diphtheria carrier, nevertheless, is an important economic factor today. Some idea of this importance can be gauged when the burden which each diphtheria carrier lays upon the Metropolitan Asylums Board, and thus on the general population of London, is considered. The cost for each patient under the Metropolitan Board is over £4 per week. Each carrier, therefore, costs at least £4 every week he stays in hospital. I have had under my care at the North-Eastern Hospital as many as 100 carriers at a time. Each of these carriers had been in hospital at least twelve weeks, and many for much longer periods. They were thus costing the community at least £400 per week as long as they remained in hospital. It must be remembered that these figures apply to only one group of fever hospitals, and that to estimate their full significance, they must be applied to the country as a whole.

It will be clear then that even from a purely economic

standpoint, the consideration of the diphtheria carrier is justified, and, in consequence, the amount of work carried out on an investigation and the expenses incurred, must be amply repaid.

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THE PROBLEM.

The following pages show the arrangement and scheme of work originally submitted by Dr. McCartney, Director of Research and Pathological Services, to the Scientific Advisory Committee of the Metropolitan Asylums Board. It is along these lines that my work in the present investigation has proceeded. As the investigation continued, its scope has been greatly increased, and many new avenues have been opened up, but since the original scheme forms the basis of the whole investigation, it will now be stated.

It has been definitely established that diphtheria bacilli tend normally to disappear during convalescence, and as the foundation of this work it has been assumed that the carrier state is a failure of a normal process of elimination.

The methods by which bacilli are removed normally during convalescence may be divided into three groups:-

1. Mechanical.
2. Chemical.
3. Biological.

(1). MECHANICAL: The mechanical action of the musculature of the tongue and pharynx in removing all foreign bodies, including bacteria, from the throat is well-known and needs no explanation.

In this way many bacteria are removed before they can obtain a hold upon the mucous membrane.

(2). CHEMICAL: The secretions of the upper respiratory passages probably have some action upon the bacteria which tend to invade that region. Thus it has been shown that the saliva has a germicidal effect upon certain bacteria.

(3). BIOLOGICAL: This factor may operate in one of two ways. In the first place, the effect of phagocytosis in the elimination of bacteria is often considerable, and must be remembered. Secondly, there is the possibility that the normal flora of the upper respiratory passages may exert a pronounced influence upon any foreign bacteria.

This latter factor appeared to be one which required further study, and one in which the possibility of success therefrom appeared considerable.

A series of very illuminating papers on this whole question has been published by Bloomfield. These papers deal with the natural flora of the mouth, and also with the power of the protective mechanism of the upper respiratory passages to cope with any extraneous organisms which may find their way either casually or through a carrier condition into that region. In a paper(77) dealing with the bacteria usually found in the throats of healthy people, Bloomfield finds that the flora of the throat falls into two classes:-

(1). The true normal flora, including non-haemolytic streptococci and Gram-negative cocci.

(2). Pathological and non-pathological organisms which are accidentally introduced, and are present only for a short time in a given individual.

In a further paper[78] dealing with the dissemination of bacteria in the upper respiratory air passages, he describes the result of experiments carried out with carbon particles and charcoal and also with sarcina lutea. He concludes that, "The experiments described in this paper and in the preceding one show clearly that there is a definite mechanism whereby foreign organisms which enter the mouth are removed. Its essential feature is a direct and rapid transport of the bacteria towards the aesophagus. Without having further experimental proof it appears probable that the organisms are swallowed after reaching this point. It does not seem wise at present to go too far in ascribing a purposeful significance to this mechanism of elimination". He also states that, "The most significant finding, however, is in connection with the tonsils. Our idea had been, (and we believe it to be generally held), that these structures were a collecting place for foreign bacteria which enter the mouth. The present experiments show that the tonsils are very well protected from contamination because of their situation behind the anterior pillars, and

because of the course of the suction currents, but that if, in some way, foreign particles do lodge on them such particles remain stagnant for a considerable length of time". He continues this study under two further papers(79), and his findings here are that, "An analysis of the possible factors active in effecting this disposal indicated that reaction of mouth secretions, mechanical action and other mouth bacteria play little, if any, part, but that the saliva and mouth secretions exert a prompt and marked bactericidal effect." Finally, he reports on an investigation into the carrier state with reference to Friedländer's bacillus(80), and finds that the breeding place of that organism is the tonsil. He further proves that the Friedländer's bacillus when sown on the free surface of a mucous membrane disappears at the same rate irrespective as to whether or not the case is a carrier. In addition, he was unable to produce a carrier state artificially with repeated inoculation of Friedländer's bacillus. His final conclusion is that, "The carrier state depends on a focus of diseased tissue which affords a breeding place for the bacteria. The organisms do not become adapted to grow on the free surface of the mucous membrane".

These papers are quoted somewhat fully as they have a direct bearing upon the present investigation, while the standard of work done was such as to render the conclusions

arrived at very valuable.

If the normal mechanism for removing organisms was in order, however, several reasons might still exist to explain the persistence in the upper respiratory passages of organisms foreign to that region. Thus, this persistence might be due to:-

(1). The presence in the upper respiratory passages of pathological processes, such as septic tonsils, enlarged adenoids, enlarged turbinates, catarrh of the nasal mucous membrane, or indeed any departure, however slight, from the normal.

(2). The presence of anatomical anomalies. These might include a cleft palate, hare lip, or any other congenital defect of the upper respiratory passages.

(3). A variation in the biological activities of the micro-organisms concerned, allowing them to assume acquired, or to exercise inherent, qualities for adaption.

It seemed necessary, therefore, to examine any pathological process or anatomical abnormality such as have been detailed above.

In addition, a series of questions was drawn up. These questions presented themselves naturally for examination, and they are given now since they represent the salient features of the scheme as they appeared at the commencement

of the investigation.

They are as follows:-

(1). Is the case a true carrier of virulent diphtheria bacilli?

(2). Is the carrier state constant or intermittent?

(3). Does the usual throat or nose flora of a carrier differ from that of a normally recovering case or of a healthy person?

(4). Is there any pathological process which will harbour bacilli, from which these are continually being introduced into the mouth?

(5). Is the intermittency, if any, pari passu, with any change in a pathological process?

(6). If a pathological process exists, is it always in the same site in all carriers, e.g. only in the tonsil, or may it occur elsewhere, in the adenoids, turbinates, etc.?

(7). Is the carrier state purely a feature of the micro-organism, i.e. does the organism become one of the normal flora without a pathological process?

(8). If (7). is correct, will a carrier strain of diphtheria bacilli produce a carrier strain in a negative recovered case?

(9). Does the carrier state arise from a lack of protective

substances in the blood, for example, antitoxins, bacterial anti-bodies?

To summarise, it will be sufficient to say that the work was concerned with discovering the reason why diphtheria bacilli should persist in carriers. When that had been established, and not until then, the question of a suggested form of treatment and possible cure could be dealt with.

These then were the problems and scheme of work submitted to me. The following pages embody a detailed description of how this work was carried out and enumerate the results and conclusions obtained therefrom.

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THE INVESTIGATION.

THE PRELIMINARY WORK.

INTRODUCTORY.

When I started work at the North-Eastern Hospital, there were collected in the hospital some 30 carriers. These were entirely unknwon to me, and so my first duty was to establish the presence of the carrier condition in these patients.

As the investigation was only commencing, it was decided that each case should be examined in detail over a period of at least eight weeks. This was done, and at the end of eight to ten weeks a set of results had been obtained showing exactly where, and to what extent, diphtheria bacilli were present in each case.

In the early part of the work the carriers dealt with comprised those cases who had become carriers, as already defined, while at the North-Eastern Hospital. Later, the carriers from all the Metropolitan Asylums Board Infectious Diseases Hospitals were transferred to the North-Eastern Hospital, and came within the scope of the present investigation. This meant a very large influx of carriers, and it was then impossible to allot a period of eight weeks' probation to each case, since by this time various forms of treatment were being

tried, and it was not considered justifiable to keep a child or an adult in hospital for eight weeks without giving them the benefit of even a trial form of treatment. In addition, the cases transferred had been carefully investigated before reaching the North-Eastern Hospital, and, in most instances, the notes supplied from the original hospital yielded full information as to the place and extent of carrying, with reports of any virulence tests which had been performed.

Full details of all the cases dealt with will be found in the appendix. These data include details as to the age incidence, sex, position of carrying, etc. of the carriers. Whenever the figures have any direct bearing upon the work done they are included in the main part of the thesis.

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THE COLLECTION OF MATERIAL.

At the outset of the investigation it was decided to take cultures from the carriers at bi-weekly intervals. The nose cultures were taken by means of a platinum loop, so as to obtain a piece of mucus or secretion from some point reasonably remote from the anterior nares, while the throat cultures were obtained from the tonsillar surface by means of an ordinary "throat swab". These "swabs" were of the usual type with the stem made either of wood or of metal. The latter was found more efficient as the wooden stem occasionally broke in the mouth of a refractory child. Wooden or metal tongue depressors were used in taking the throat swabs, and also a lamp and forehead mirror where deficient illumination rendered this necessary. In an ordinary case, cultures were made from both nostrils and from the throat. Ear cultures were taken by means of a slender type of "throat swab".

The material, (which was obtained by myself in all cases), was at once inoculated on the selected media. The media used was the ordinary Loeffler's inspissated serum which was made for hospital use by the Belmont Laboratories, Surrey. The attention of the sisters and nurses in charge of the wards was drawn to the fact that no gargles, douches or other form of

antiseptics should be given to any carrier within twelve hours of having a culture taken. The cultures were incubated for twenty-four hours at 37°C. If a negative result was obtained after examination of any culture, more material was immediately obtained from that case, and the same routine was carried out. If a special investigation of any case was desired, material was obtained from the tonsillar crypts by means of a suitably bent platinum loop. For ordinary purposes, however, this procedure, which is often terrifying, though not painful, to young children, was not adopted.

During the course of the investigation several important modifications were made in this scheme. Thus, after the investigation had been in progress for six months and carriers began to accumulate, I found it impossible to take cultures from every carrier twice a week. By this time, however, I had accurately ascertained the type of carrier which was being dealt with, and found it unnecessary to take cultures more than once a week, Monday being chosen as the most suitable day.

It must be remembered in this connection that an additional check upon the cultures was present, as the results of the ordinary bi-weekly routine ward cultures were always available.

Another economy of time was also effected about the same period with, I believe, little, if any, loss in efficiency. This comprised a change in the manner of taking the nose

cultures. Instead of taking the cultures from each nostril every week, I decided that it would be quite sufficient to take cultures each week from alternate nostrils. This meant a very large saving in time and also in media. The effect upon the results was apparently nil, as few cases were found to be harbouring bacilli in one nostril only. If special investigation as to the exact site of carrying was found to be necessary in any case, cultures were taken from each nostril on every occasion.

About this time another interesting fact was observed. It has been the custom for most authorities on this subject to state that diphtheria bacilli will grow on Loeffler's medium within twenty-four hours, and that if no growth is obtained within that time, further change is unlikely. Indeed, colonies of K.L.B. are usually stated to appear within eight to twelve hours after inoculation upon the medium. During the course of this investigation I found that many Loeffler slopes which had been inoculated in the usual manner showed no growth after twenty-four hours incubation. The incubator and the medium both appeared to be above reproach, and the cultures were accordingly allowed to incubate for a further twenty-four hours. It was then observed that a large number of Loeffler slopes which had shown no growth after twenty-four hours yielded a positive culture after forty-eight hours

incubation. This has proved the case throughout the remainder of the investigation, and the following table is given to show the percentage of positive cultures obtained after the second day's incubation.

Date of Examination of cultures.	No. of Cultures incubated.	No.and percentage of "positives" after 24 hours incubation.	No.and percentage of additional "positives" after 48 hrs.incubation.
19.4.1927.	100.	15 - 15 per cent	11 - 11 per cent
26.4.1927	98	13 - 13.3 "	10 - 10.2 "
2.5.1927	78	8 - 10.3 "	11 - 13.1 "
9.5.1927	81	13 - 16 "	9 - 11.1 "
16.5.1927	85	16 - 18.8 "	8 - 9.4 "
23.5.1927	95	7 - 7.4 "	8 - 8.4 "

To make sure that the medium was not at fault, special batches of media were prepared at the Northern Group Laboratory under particularly careful conditions. These yielded practically identical results. During the remainder of the investigation use was made of this fact. That is, instead of obtaining material from negative cases on the following day, negative cultures have been allowed to incubate for a further twenty-four hours, and then examined. This appears to be quite satisfactory,

yields at least as many "positives" as the previous method, and saves a vast amount of time and labour.

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THE EXAMINATION OF THE MATERIAL OBTAINED.

The examination of the cultures did not present any special difficulties. Smears were made from each culture, stained, and examined in the usual way. The smears were obtained by emulsifying the whole culture in the water of condensation, and then taking a large loopful of the suspension so procured. Thus a representative smear was obtained, and so the likelihood of a few colonies of K.L.B. being missed was lessened. Films were stained by Pugh's method. This yielded quite satisfactory results. Later, however, a stain which had as its basis Toluidine Blue and Azur-"L"* was substituted. This modification brings out a clearer distinction between the granules and the bodies of the bacilli and considerably facilitates the microscopical examination of films. All doubtful bacilli in twenty-four cultures were classed as "negative" and re-examined after a further twenty-four hours' incubation.

* This is an oxidation product of Toluidine Blue, and was made specially by G.T. Gurr at the suggestion of Dr. J.E. McCartney.

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THE VIRULENCE TEST.

I have stressed the importance of the virulence test several times, and now I wish to give in some detail the method used to carry out the test.

When the investigation commenced, it had already been proved that several cases were harbouring virulent diphtheria bacilli. It was necessary in most cases, however, to carry out a virulence test for each carrier, and an attempt was made to establish the virulence of every case considered under this investigation. This, however, was found to be impossible for several reasons. In the first place, not a few cases after being positive for twelve weeks and coming under my care, ceased to carry and showed an entire series of negative cultures. These cases mainly consisted of intermittent carriers who were just commencing to clear up when they became official "carriers". If a virulence test had not been performed previous to their being carriers, it was obviously impossible to carry out one later. Again, many cases only showed one or two very scanty positive cultures after officially becoming "carriers", and it was often quite impossible to isolate these organisms for the virulence test. The number of virulence tests performed, and the results obtained, will be fully considered when the final

data come to be dealt with.

At the start of the investigation and for the first six months I carried out the virulence testing myself. Later, when the Northern Group Laboratory opened officially in May, 1926, and the virulence tests for all the hospitals under the Metropolitan Asylums Board were performed there, the work was first done by myself and later by Dr. W. Mair, Assistant Director of Research and Pathological Services.

The routine method for the virulence test used in the Research and Pathological Services of the Metropolitan Asylums Board is as follows:-

A fairly thick emulsion of the culture to be tested is made in normal saline. A loopful of this emulsion is inoculated on a tellurite-trypsin-serum-agar plate. This particular medium was first introduced by Douglas(81), and later modified by Mair and Ayling(82). The latter authors summarise the benefits to be obtained by its use as follows:-

"The natural antitryptic power of the serum is partially neutralised by the addition of trypsin; this greatly increases its value as an enrichment of the ordinary nutrient agar for the growth of many bacteria, and, in particular, for the B.diphtheriae; the addition of potassium tellurite to the medium inhibits the growth of the various other bacteria more than it does that of B.diphtheriae, and, what is more important, the blackening of the tellurite in different degrees by the

various bacteria gives rise to colonies which are more or less characteristic of each species. Thus it is comparatively easy on this medium to pick out colonies of the diphtheroid group (including B.diphtheriae) for purposes of isolation in pure culture".

It is thus possible to pick off a suspicious colony after twenty-four hours' incubation and to reject entirely a plate which does not appear to show a colony of the typical diphtheria type. This is usually impossible when using Loeffler's medium or the ordinary agar or serum-agar. The diphtheria colony appears on the tellurite medium as a smooth, round, dull black colony. The colony is surrounded by a pale greyish transparent border. There is occasionally umbilication at the centre of the colony, but in most cases the surface is quite smooth. Colonies of cocci usually show as brilliant black, circular colonies with a shining surface. Colonies of diphtheroids present all varieties between these two types. Occasionally, aberrant and mixed forms occur, and it is sometimes impossible to distinguish a colony of K.L.B. from a colony composed of cocci or other organisms. The selective properties of this medium, however, make it a very valuable adjuvant in the attempt to isolate the diphtheria bacilli in pure culture. The illustrations appended at the end of the thesis show the usual types of colonies found.

Having inoculated tellurite-agar plates with the culture

to be tested, colonies of organisms morphologically resembling B.diphtheriae are picked off the plates and subcultured on to serum-agar or plain agar slopes. (For this purpose a low-power binocular plate microscope (Leitz) is used, giving a magnification of ten diameters. This greatly facilitates the process of isolation). A portion of the pure culture so obtained is then inoculated into tubes of Hiss serum water containing glucose and saccharose in a concentration of 0.5 per cent, and Andrade's (neutral) indicator. The presence of fermentation is shown by the acid turning the straw-coloured indicator bright red and by the coagulation of the medium. The diphtheria bacillus ferments glucose with acid production, but has no effect upon saccharose. Thus the glucose solution turns bright red, while the saccharose solution remains unchanged. A saccharose-fermenting diphtheroid produces acid in both glucose and saccharose with consequent pink colouration in both tubes. A Hofmann strain, on the other hand, produces no fermentation and so causes no change in either sugar.

Having obtained a pure culture of an organism morphologically resembling the Klebs-Loeffler bacillus which ferments glucose but not saccharose, the actual virulence test is then carried through. The test culture used is one obtained after twenty-four hours' growth on serum agar. An emulsion of this

culture having a just visible opacity is made in normal saline. The method adopted is that generally known as the "percutaneous method". This method was first suggested by the work of Fritsche[83], in 1902. It was elaborated by Römer[84] in 1909, and later modified by Neisser and Gins[85], in 1913, and by Zingher and Soletsky[86], in 1915. More recently Eagleton and Baxter[87], in 1921, have described a technique modelled on these lines but with some improvements. A modification of the method of these last observers is employed as follows:-

Two guinea-pigs of about 400 grammes in weight are selected. These should be completely white or at least predominantly so. The guinea-pig which is to be used as the control is given 1,000 units of diphtheria antitoxin intraperitoneally twenty-four hours before the virulence test is to be performed. This should entirely prevent the appearance of the typical cutaneous reactions produced by the injection of the diphtheria bacilli. The skin on the flanks of both guinea-pigs is depilated on the morning of the test. For this purpose, a mixture of hard soap, talc powder and barium sulphide is used. 0.2 cc. of the emulsion of the organism to be tested is then injected into the depilated skin. Ten or twelve tests can be made on each guinea-pig with perfect safety. The injections are made first in the guinea-pig which is to be used

for the virulence test (known as the "test guinea-pig), then the same is repeated in the "control guinea-pig". About four and a half hours later, the "test guinea-pig" is given a "following dose" of diphtheria antitoxin amounting to 250 units. This is sufficient to save the life of the guinea-pigs without obscuring the readings in any way. A positive reaction is indicated by reddening and swelling which appear around the sites of injection within twenty-four hours. This usually proceeds to ulceration and necrosis within forty-eight to seventy-two hours, and if left alone ultimately heals with the production of a cicatrix. A negative result is shown by a complete absence of reaction after twenty-four to forty-eight hours, or at most only a slight reddening which disappears after forty-eight hours. In addition, the control animal should show a complete absence of reaction (the illustrations at the end of the thesis show typical positive and negative reactions, with the corresponding controls). This test is extremely delicate. The amount of antitoxin given must be carefully judged and can best be gauged by experience. If too little antitoxin is given to the "control guinea-pig" before the test, the reactions on the control animal may show to the same extent as on the "test guinea-pig". In addition, if the "following dose" of antitoxin given to the "test guinea-pig" is too small the guinea-pig may die before the readings can be

ascertained. It is seldom necessary to use a known virulent or non-virulent culture as a control in the series unless the cultures to be tested are known to be of doubtful virulence. The result of the test can be provisionally read within twenty-four hours, and a final reading obtained within forty-eight hours. The animals are usually killed after the test, but, occasionally, when the supply runs short, the "control guinea-pig" is used a second time. This does not appear to affect the results in any way.

Before finally adopting this method, the technique was thoroughly tested out against the older subcutaneous method, and the results were found to tally exactly.

* * * * * * *

The foregoing description of the preliminary work has been given in detail as it forms the basis of all the future scheme of investigation. This part of the work took several months to complete, as it was felt necessary to become familiar with the type of carrier and the type of organisms being dealt with, before any more definite steps could be taken. Every carrier was thus known very thoroughly, and the site of the organism and its virulence established before any further line of action was decided upon. If the carrier condition cleared up during this stage, or even showed signs of clearing up, nothing

further was done. If, however, the cultures persisted strongly positive during the preliminary stage then the carrier remained an important subject for further investigation, and was dealt with accordingly.

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THE CARRIER PROBLEM.

SUMMARY OF THE WORK DONE.

The following is a short summary of the work carried out in the chief portion of this investigation.

(1). 350 diphtheria carriers were investigated over a complete period of two years. These cases were all reputed "chronic carriers" of at least twelve weeks duration, and were chosen irrespective of age, sex, or type of original disease. Complete investigation of the general physical condition of the carriers, type of original disease, etc. was made, and the cases were kept under constant bacteriological and clinical supervision. 7 of these cases were proved to be carrying non-virulent organisms. In 16 cases the preliminary period of twelve weeks could not be vouched for, and so these cases were omitted from consideration. The total number of "chronic carriers" of true diphtheria bacilli dealt with was, therefore, 327.

(2). The bacterial flora of the nose and throat of carriers, of convalescent diphtheria patients, and of apparently normal school-children was thoroughly investigated.

(3). Clinical examination was made of the noses and throats

of the carriers; of 300 convalescent diphtheria patients, four to five weeks after admission; and of 100 diphtheria patients, immediately before discharge.

(4). Autogenous vaccines were prepared and administered to 48 carriers. The vaccines were composed of all the types of organisms found in the noses of the carriers treated. The effect of the vaccines was checked by the examination of blood plates inoculated with material from the noses of the carriers under treatment, to ascertain whether or not the organisms had disappeared from the nose.

(5). 63 carriers were treated by alkaline nasal douching. Here again blood plates were made and examined.

(6). 68 cases were treated by the removal of tonsils and adenoids. Bacteriological examination of all the material obtained at operation was undertaken, the presence of K.L.B. being searched for carefully. Sections were cut and stained from all the specimens obtained.

(7). (a). The antra of Highmore of 3 carriers were explored.

(b). 2 carriers were treated by mastoidectomy.

(c). 4 carriers were treated by spraying the nose with iodine oil.

(8). The resistance of the carriers was estimated by carrying out a Schick test on each case. In several cases, an

attempt was made to discover the presence and to estimate the amount of specific agglutinins and complement in the blood.

(9). Virulence tests were performed wherever possible and repeated where necessary.

The following pages embody a detailed description of the work done upon the carriers.

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(A). THE INVESTIGATION OF THE BACTERIAL FLORA OF CARRIERS, DIPHTHERIA PATIENTS, AND APPARENTLY HEALTHY SCHOOL-CHILDREN.

The possible effect which the composition of the bacterial flora of the nose and throat might have upon the carrier condition has already been noted. This question, therefore, received early attention, and a scheme was drawn up whereby sufficient data might be obtained for consideration.

THE THROAT FLORA.

The first site to be investigated was the throat. 13 strongly positive carriers were selected for investigation. Material was obtained from the throats by swabbing, and this material was inoculated on blood-agar plates. These blood plates were made by preparing a saline suspension of the material obtained by swabbing, and mixing various quantities of that suspension into the blood-agar, thus producing a "poured plate". One, two or three plates, as considered necessary, were made from each case at each examination. Blood plates were made from all cases at frequent intervals until enough material had accumulated for the flora of each case to be accurately estimated. In this way several hundred blood plates were examined from the carriers selected.

While the flora of the selected carriers was being examined, a series of diphtheria cases was taken and the throat flora

examined in the same manner. Material was obtained for making blood plates on the day of admission, and the plating was continued at bi-weekly intervals until the patient was discharged. Nose and throat cultures were taken simultaneously and examined for the presence of K.L.B. 15 diphtheria patients were dealt with in this way, and altogether at least 220 blood plates were made and examined. There were quite a number of difficulties met with in this work, as only a small proportion of diphtheria cases on admission were in a state which allowed of swabs being taken. Indeed, in all cases, great care had to be exercised in the swabbing.

In addition, an arrangement was made with the London County Council whereby I was allowed to visit Duncombe Road Schools, and swab the throats of 55 apparently healthy schoolboys. Blood plates were made from the swabs, and so a group of 110 plates was obtained for comparison.

The great bulk of the carriers examined were children of between five and fifteen years of age. The diphtheria patients and the children were, therefore, chosen from children of about the same age, so that the age composition of each group was as nearly as possible the same.

RESULTS.

The results obtained from this part of the investigation were briefly as follows;

There did not appear to be any direct relationship between the flora of the throat of carriers and the presence of the carrier state. This conclusion was arrived at from a close study of the results obtained from the examination of the blood plates in the three groups of cases already mentioned. Thus it was found that in any of the groups selected the same organisms appeared with unfailing regularity. No organisms appeared to be confined to one group while being absent from the others. Staphylococcus (aureus and albus), streptococcus (haemolytic and "viridans"), B.influenzae (Pfeiffer), micrococcus catarrhalis and pneumococcus composed the flora in the great majority of the throats examined. This applied equally to carriers, convalescents and school-children. Diphtheria cases on admission showed a more extensive flora than did the other groups of cases, but considering the inflamed condition of the throat on admission, this did not appear surprising. The plates made from the throats of the school-children were very similar to those made from the throats of the carriers examined.

As regards the carriers, there did not appear to be any organism invariably present in, or absent from the flora of the throats examined, nor did the flora change greatly if the case showed signs of clearing up. Similarly, the flora of acute diphtheria cases remained relatively unchanged right throughout

convalescence, irrespective of the severity of the original disease or of the length of convalescence. The following shows the type of organisms found in each group:-

BLOOD PLATES (THROATS).

Type of Organisms	Persistent carriers.	Intermittent carriers.	Control school-children. Normal throats.	Acute diphtheria cases.
Beta haemolytic Streptococcus	Fairly constant	Occasionally present	Fairly constant but usually few	Fairly constant.
Alpha prime haemolytic Streptococcus.	Fairly constant	Fairly constant	Fairly constant	Fairly constant.
"Viridans"(Alpha haemolytic) Streptococcus	Constant	Constant	Constant	Constant.
Non-haemolytic Streptococcus.	Rarely present.	Rarely present	Rarely present.	Fairly constant.
Pneumococcus.	Occasionally present	Occasionally present	Not very often present.	Varies according to type of case
"Haemophilic group"	Rarely present	Rarely present	Rarely present	Rarely present.
Staphylococcus albus	Occasionally present	Occasionally present	Occasionally present	Occasionally present
Staphylococcus aureus	Never present	Never present	Never present	Rarely present
"Gram negative cocci"	Fairly constant	Fairly constant	Fairly constant	Fairly constant.
Friedländer's bacillus	Rarely present	Rarely present	Rarely present	Rarely present.
Diphtheroids	Never present	Never present	Never present	? Never present.
Klebs-Loeffler bacillus	Quite commonly present	Rarely present	Never present	Present Diminished during convalescence

N.B. The "viridans" group of streptococcus was differentiated from the pneumococcus by means of the bile solubility test, using desoxycholic acid, as described by Mair (88).

It could not be stated, therefore, that any particular organism found in the throat was exercising a beneficial or an antagonistic influence on the Klebs-Loeffler bacillus.

The graphs appended at the end of this thesis show the apparent lack of relationship between the results of the cultures as regards the presence of K.L.B. and the type of organisms present in the throat.

THE NOSE FLORA.

The flora of the nose next received attention. The procedure was the same as that carried out in the examination of the throat flora. In this case, however, the results obtained varied somewhat from those of the first series. The broad result of the examination of the blood plates made from the noses of carriers, diphtheria convalescents and school controls was to show that the flora present in the noses of carriers was much more extensive than that found in the other two types of cases examined. The flora did not differ much in the three groups as regards the type of organisms found, but quantitatively there was considerable variation. The nose flora of the carriers examined was exceedingly proliferaht. Very few clean or moderately clean noses were

found. The school controls, on the other hand, yielded quite different results. Of the 50 children examined, 10 showed no growth on the blood plates, while 24 yielded plates with only scanty growth, 13 of them being practically sterile. A few cases were obtained in which a luxuriant flora was present, but the existence of "common colds", "running noses", etc. could never be quite excluded, especially as the work was done during a rather cold season. On several occasions most of the boys examined in one class showed the presence of Beta haemolytic streptococcus in their plates. This appeared to be an interesting example of a "class infection". As all the persistent and most of the intermittent nose carriers had their flora examined, and as the examinations were repeated many times, the number of plates investigated was exceedingly large and appeared quite sufficient to warrant a definite statement being made upon the result.

The following tables show the relative constitution of the flora of the three groups investigated, together with the results of several representative cases and groups of cases.

TABLE I.

BLOOD PLATES (NOSES).

Type of organisms.	Persistent carriers.	Intermittent carriers.	Control school-children Normal noses.	Acute diphtheria cases.
Beta haemolytic Streptococcus	Fairly constant	Fairly constant	Occasionally present	Rarely present
Alpha prime haemolytic Streptococcus	Fairly constant	Occasionally present	Never present	Rarely present.
"Viridans" (Alpha haemolytic) Streptococcus.	Occasionally present	Occasionally present	Rarely present	Occasionally present
Non-haemolytic Streptococcus	Rarely present	Rarely present	Rarely present	Occasionally present
Pneumococcus	Rarely present	Rarely present	Rarely present	Rarely present
"Haemophilic group"	Occasionally present	Rarely present	Never present	Rarely present.
Staphylococcus albus.	Constant	Constant	Often present	Constant
Staphylococcus aureus.	Constant	Constant	Rarely present	Rarely present.
"Gram negative cocci"	Occasionally present	Rarely present	Never present	Rarely present
Friedländer's bacillus	Rarely present.	Never present	Never present.	Rarely present.
Diphtheroids	Occasionally present	Occasionally present	Never present	Rarely present.
Klebs-Loeffler	Commonly present	Occasionally present	Never present	Occasionally present. Diminishes during convalescence

TABLE II.

(1). Case No.44. Persistent nose carrier. Blood plates made from the nose on 8 occasions.

Results. Beta haemolytic streptococcus present on 3 occasions.
Alpha haemolytic streptococcus present on 1 occasion.
Staphylococcus albus present on 6 occasions.
Gram negative bacillus present on 3 occasions.
K.L.B. present on 3 occasions.
Blood plates sterile on 1 occasion.

(2). Case No.52. Persistent nose carrier. Blood plates made from the nose on 8 occasions.

Results. Beta haemolytic streptococcus present on 2 occasions.
Alpha haemolytic streptococcus present on 1 occasion.
Staphylococcus albus present on 7 occasions.
Staphylococcus aureus present on 3 occasions
Gram negative bacillus present on 1 occasion.
Blood plates never sterile.

(3). Results obtained from the examination of blood plates made from the noses of 10 boys attending one class at Duncombe Road Schools - 16.9.1926.

Results. 6 plates sterile
1 plate practically sterile.
Staphylococcus albus present in 3 cases.
Non-haemolytic streptococcus present in 1 case.

(4). Case 10X. (Nasal and faucial diphtheria). Blood plates made from the nose on 7 occasions.

Results. Beta haemolytic streptococcus present on 1 occasion.
Non-haemolytic streptococcus present on 5 occasions.
Staphylococcus albus present on 4 occasions.
K.L.B. present on 1 occasion.
Blood plates never sterile.

(5). Case 11X. (Clinical diphtheria, nose and throat positive) Blood plates made from the nose on 6 occasions.

Results. Beta haemolytic streptococcus present on 1 occasion.
Non-haemolytic streptococcus present on 2 occasions.
"Viridans" present on 1 occasion.
Staphylococcus albus present on 4 occasions.
Staphylococcus aureus present on 1 occasion.
Blood plates sterile on 1 occasion.

(6). Case 15X. (Nasal and faucial diphtheria). Blood plates made from the nose on 15 occasions.

Results. Beta haemolytic streptococcus present on 8 occasions.
Non-haemolytic streptococcus present on 5 occasions.
Staphylococcus albus present on 12 occasions.
Friedländer's bacillus present on 1 occasion.
Gram-negative coccus present on 4 occasions.
K.L.B. present on 6 occasions.
Blood plates sterile on 2 occasions.

The cases enumerated above represent the type of organism isolated in each group dealt with. Exceptions were found, but, on the whole, these cases are fully representative of the group to which they belong.

I also examined blood plates inoculated with material obtained from chronic carriers and incubated under anaerobic conditions. I controlled these by incubating aerobically plates made from the same cases. The results were practically identical, the same flora being present in the two sets of plates. Thus it did not appear that any anaerobic organism was playing an important part in sustaining the carrier condition.

CONCLUSIONS. From the examination of the flora of carriers and controls, it appeared quite definite that the nose flora of "chronic carriers" differed considerably from that of non-carrying members of the community of roughly the same age. This fact having been established, a further line of investigation was suggested. If diphtheria carriers harboured a much more abundant nose flora than did non-carriers, it appeared to be necessary to try the effect upon the carrier state of decreasing this flora. Various measures of carrying this out presented themselves, as for instance:-

(1). The use of autogenous vaccines made from the organisms found in the noses of the carriers concerned.

(2). The douching of the nose with an alkaline douche.

(3). The application of various antiseptics to the nasal mucous membrane.

It seemed wise to concentrate upon the nose flora, as the material obtained up to this time had established the fact that the important carrier was the "nose carrier". It was comparatively easy, as will be shown later, to deal with the "throat carrier", the results of the removal of tonsils and adenoids in these cases being eminently satisfactory. In addition, the examination of the throat flora had not yielded any very useful information of a positive nature.

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(B). THE CLINICAL EXAMINATION OF CARRIERS AND CONTROLS.

Before dealing with the various attempts to influence the nose flora of carriers I wish to discuss another factor of correlative importance. When the investigation commenced it was decided to examine the throats and noses of all the carriers from a clinical standpoint, to see if any abnormality or pathological condition which might be exerting an influence upon the carrier condition was present. For this purpose, Mr. Humphrey Marriner, Clinical Assistant at St. Thomas's Hospital, was engaged by the Metropolitan Asylums Board. Mr. Marriner attended the North-Eastern Hospital once, and later, twice a week. We visited the wards together, and carried out the necessary clinical examinations. Any desired form of treatment was mutually decided upon, and, where necessary, Mr. Marriner performed the operations. These included the removal of tonsils and adenoids, and the exploration and cleaning out of antra.

The main features which were looked for in the examination of the carriers and controls included recognisable abnormalities of the nose, naso-pharynx, throat and ears. As the great majority of the cases were children under five years of age the difficulties encountered were considerable. Any abnormality found, however slight, was classed as a pathological condition,

as it was not known how far such might influence the flora of the region concerned, and so play a part in inducing or sustaining the carrier condition. These abnormalities included crusting of the nasal mucous membrane, enlarged or engorged turbinates, deflected nasal septa, and enlarged tonsils, even though these tonsils were apparently healthy. The following chart is a copy of the type which was drawn up and used during the investigation;-

Name_____ Age_____ Sex_____ Admitted_____

Type of case on admission_____

Oto-rhino-laryngological Examination.

Appearance.

Ear: R. / L.

Pharynx: (R.Tonsil (L.Tonsil (Pharynx.

Nasopharynx.

Nose: (R,Nostril (R.Inf.turb. (R.Mid.turb. (L.Nostril (L.Inf.turb. (L.Mid.turb. (Septum.

Adenoids Adenitis R. / L.

Operation_____ Date_____

310 carriers were examined in this way, and the results obtained were very illuminating. When, however, the established facts were communicated by Dr. McCartney to the Scientific Advisory Committee, the suggestion was made that a similar number of diphtheria patients should be examined in a like manner, in order to obtain controls. 300 diphtheria patients were, therefore, examined four to five weeks after their admission to hospital, and the results recorded. Later, a further suggestion was made that a number of diphtheria patients should also be examined immediately before discharge. 100 cases of this kind were, therefore, chosen. Three groups of results were then available for comparison.

It must be realised that several cases were represented in two, and some even in three groups. This would occur when a case was examined four weeks after admission, became a carrier and was examined as such, and finally was examined on discharge. These cases, however, do not alter the value of the figures in any way. The age composition of the three groups was not specially selected, as I thought it best to have all the cases examined strictly as they arose. Nevertheless, the age distribution of the three groups corresponded reasonably closely, except that there were relatively more carriers in the 1 - 5 years group than were there diphtheria patients or discharges in the same category.

CLINICAL EXAMINATION OF CARRIERS.

Among the carriers examined, the majority displayed pathological conditions present both in the nose and throat. A small proportion showed lesions in the nose or throat only, while a very small proportion showed no apparent abnormalities on examination.

The following table shows the complete results in detail:-

Details of 288 cases.

No. of cases in which pathological conditions were found present in the throat only		63
No. of cases in which pathological conditions were found present in the nose only		67
(No. of cases in which pathological conditions (were found present in right side of nose	12	
(No. of cases in which pathological conditions (were found present in left side of nose	0	
(No. of cases in which pathological conditions (were found present in both sides of the nose ..	55	
	67	
No. of cases in which pathological conditions were found present in the nose and throat		141
No. of cases in which pathological conditions were found present in the ear only		2
No. of cases in which no pathological abnormalities were found		15
		288

TABLE SHOWING DISTRIBUTION OF PATHOLOGICAL CONDITIONS ACCORDING TO AGE GROUP.

Age.	Throat only affected.	Nose only affected.	Throat and Nose affected.	Nothing abnormal found.
Under 1 year	1	3	2	1
1 - 5 years	36	37	98 (one cleft palate)	8
5 -10 "	18	20	32	5
10 -15 "	7	5	5	1
Over 15 "	1	2	4	-
Totals	63	67	141	15

= 286 cases

N.B. The ages of the patients over 15 years of age were as follows:-

18, 18, 19, 23, 20, 27 and 28 years.

The 2 cases in which the ear only was affected are omitted from this table.

There were no marked differences in the data obtained obtained from persistent and from intermittent carriers.

In addition, I estimated the duration of the carrier state, classifying the cases as regards the presence of pathological conditions in the nose and throat. The results were as follows:-

Average duration of the carrier state in cases where the nose only was clinically affected .. 24.3 weeks

(Average duration of the carrier state in cases where the nose was only slightly affected .. 19.5 weeks.

Average duration of the carrier state in cases where one side of the nose was affected .. 25.2 "

Average duration of the carrier state in cases where both sides of the nose were affected .. 24.5 ").

Average duration of the carrier state in cases where the nose and throat were clinically affected .. .. 23.5 "

Average duration of the carrier state in cases where the throat only was clinically affected .. 19 "

Average duration of the carrier state in cases where the ear, nose and throat were clinically affected .. .. 37 "

Average duration of the carrier state in cases where the ear and throat were clinically affected .. .. 31.1 "

Average duration of the carrier state in cases where the ear and nose were clinically affected .. .. 27.1 "

Average duration of the carrier state where nothing abnormal was found in the ear, nose or throat .. .. 18 "

Two cases were found with cleft palates. One of these cleared up within six months; the other on which an unfinished operation was performed has been a carrier for over two years

and is still positive. No form of treatment has had any effect in this case, and hospital difficulties have made the completion of the operation impossible. In two cases ozoena was present, and in each of these cases the carrier state was of very long duration. In three cases the antrum of Highmore was affected, and here again the carrier state was of prolonged duration.

Thus it will be seen that the presence of abnormalities in the nose coincides with a long duration of the carrier state, a condition of affairs which had been suspected early in the investigation. It must be remembered, however, that the effect of treatment, which was going on during this investigation, had a decided influence upon the duration of the carrier condition. This influence was exerted in the direction of reducing the period of carrying in those cases which showed abnormalities in the nose, or nose and throat, as these cases were usually the ones which were chosen for treatment. This fact, therefore, serves to emphasise the importance of the results shown in the foregoing table.

EXAMINATION OF DIPHTHERIA CONVALESCENTS.

The examination of the 300 diphtheria convalescents yielded different results. Here, the proportion of cases with pathological conditions in the nose was much smaller than among the carriers, and the proportion of cases showing no

abnormalities, much larger. The complete figures obtained from the diphtheria convalescents, arranged according to age group, are appended.

TABLE SHOWING DISTRIBUTION OF PATHOLOGICAL CONDITIONS ACCORDING TO AGE GROUP.

Age.	Throat only affected.	Nose only affected.	Throat and Nose affected.	Nothing abnormal found.
Under 1 year	-	-	-	1
1 - 5 years	28	15	31	25
5 -10 "	43	19	40	27
10 -15 "	11	5	13	14
Over 15"	9	2	3	14
Totals.	91	41	87	81.

= 300 cases.

These cases were picked indiscriminately as to age, severity of disease, etc., from the wards, but all had been approximately four weeks in hospital. They included cases of faucial, nasal, nasal and faucial, laryngeal, ophthalmic and bacteriological diphtheria.

The ages of the patients over 15 ranged from 16 to 59 years.

No affection of the ear was found in any case examined.

Several cases had had their tonsils removed previously.

This was also noted in the case of many carriers. 4 cases in one ward who were examined on the same day showed an interesting feature. 2 of these 4 cases had positive cultures and 2 were negative. Both the negative cases showed no abnormalities in the nose or throat, while the positive cases showed an affection of the throat, and of the nose and throat respectively.

In addition, 13 of the 300 cases became carriers. It is interesting to note the clinical findings in these 13 cases, which were as follows:-

(1).	Nose	slight crusting.
	Throat	tonsils unhealthy.
(2).	Nose	slight crusting.
	Throat	tonsils unhealthy.
(3).	Nose	crusts.
	Throat..	nothing abnormal found.
(4).	Nose	slight crusting.
	Throat..	tonsils ragged.
(5).	Nose	sore
	Throat..	nothing abnormal found.
(6).	Nose	fine crusting.
	Throat..	tonsils partially removed.
(7).	Nose	pus present and turbinates oedematous.
	Throat..	nothing abnormal found.
(8).	Nose	nothing abnormal found
	Throat..	tonsils unhealthy
(9).	Nose	nothing abnormal found
	Throat..	tonsils very large.

(10). Nose .. .. .. fine crusting.
Throat.. .. .. tonsils large and ragged.

(11). Nose .. .. .. crusting.
Throat.. .. .. tonsils septic.

(12). Nose .. .. .. mucus and crusts.
Throat.. .. .. tonsils large and unhealthy.

(13). Nose .. .. .. much crusting.
Throat.. .. .. tonsils very septic.

Thus in 11 out of the 13 cases pathological abnormalities were found in the nose; in 9 cases pathological abnormalities were found in the throat; in 7 cases the nose and throat were both affected, and in no case were the nose and throat both free from pathological abnormalities.

Thus it appeared as if the presence of marked pathological processes in the nose of a diphtheria patient was conducive to a carrier state supervening in that patient.

EXAMINATION OF PATIENTS BEFORE DISCHARGE.

The following table gives the results of the examination of 100 diphtheria patients immediately before discharge from hospital:-

TABLE SHOWING DISTRIBUTION OF PATHOLOGICAL CONDITIONS ACCORDING TO AGE GROUP.

Age.	Throat only affected.	Nose only affected.	Throat and Nose affected.	Nothing abnormal found.
Under 1 year	-	-	-	-
1 - 5 years	12	1	2	15
5 -10 "	21	-	2	20
10 -15 "	9	2	-	7
Over 15"	3	-	-	6
Totals.	45	3	4	48

= 100 cases.

It will thus be seen that about 50 per cent of the cases examined showed no abnormalities in either nose or throat. Of the remainder, the number of cases in which the nose was affected was exceedingly small, the great majority of the abnormalities being present in the throat. In addition, the great majority of these abnormalities consisted of large but healthy tonsils. It appears from these figures that the presence of large tonsils does not interfere with the elimination of K.L.B. from the throats of diphtheria convalescents, provided that these tonsils are not unhealthy and do not show the presence of crypts.

17 of the cases examined had been diphtheria carriers.

1 of the 100 cases had been in hospital for 4 weeks, 1 for 5 weeks, 3 for 6 weeks, 3 for 7 weeks, 43 for from 8 - 12 weeks, and 49 for over 12 weeks. Of the latter 49 cases, however, 14 had been detained in hospital for reasons apart from the presence of K.L.B. in the cultures. No affection of the ear was found present in any case examined.

Work is still proceeding on this subject, and it is hoped to gain much more material for comparison.

The following tables show the three groups of results in comparison:-

Diphtheria carriers
(288 cases).

		per cent.
Percentage of cases in which	the throat only was affected	21.9
" " " " "	nose " " "	23.3
" " " " "	nose and throat were "	48.9
i.e. " " " " "	nose was affected =	72.2
" " " " "	nothing abnormal was found	5.2
" " " " "	the ear only was affected	0.7

Diphtheria cases 4 weeks after admission to hospital.
(300 cases).

Percentage of cases in which	the throat only was affected	30.3
" " " " "	nose " " "	13.7
" " " " "	nose and throat were "	29.0
i.e. " " " " "	nose was affected =	42.7
" " " " "	nothing abnormal was found	27.0
" " " " "	the ear only was affected	0.0.

Cases ready for discharge.
(100 cases).

		per cent
Percentage of cases in which	the throat only was affected	45.0
" " " "	nose " " "	3.0
" " " "	nose and throat were "	4.0
i.e. " " " "	nose was affected	7.0
" " " "	nothing abnormal was found	48.0
" " " "	the ear only was affected	0.0.

CONCLUSIONS.

From an examination of all the material obtained it was possible to come to several definite conclusions. Firstly, it was seen that pathological conditions of the nose or throat, and especially of the nose, were commoner amongst carriers than amongst diphtheria patients during, or after, convalescence. In addition, carriers with lesions in the nose, or nose and throat, appeared more likely to have a longer stay in hospital than those who had only lesions in the throat, or whose nose and throat appeared to be normal. Indeed the length of time during which a carrier remained in hospital appeared strictly proportionate to the extent of the pathological conditions found in the nose and throat. Similarly, a diphtheria patient who showed the presence of abnormalities in the nose was apparently more likely to become a carrier than was one who did not. The clearing up of the carrier state appeared to be largely dependent upon the correction of these abnormalities. The presence of large tonsils, if the tonsils were healthy, seemed to have little

bearing upon the carrier condition.

The question of the pathological conditions and abnormalities found in the nose and throat was closely linked with that of the flora of the same regions. The presence of an increase in the flora might set up an inflammatory condition, and so give origin to a pathological process; or, on the other hand, the presence of an abnormality might provide a breeding-place for micro-organisms and so produce an increased flora. Which of the two was the usual sequence of events mattered little. The important point elicited was that the carrier condition in diphtheria was usually accompanied firstly, by an increase in the flora of the nose and throat, especially the former, and secondly, by the existence of pathological conditions and abnormalities in the same two areas. So far, the results of the investigation appeared to show that the presence of a carrier state was largely dependent upon the existence of these two conditions, and thus the carrier state did not appear to be purely a function of the micro-organism. It was, therefore, decided to try the effect upon the carrier condition of improving the general state of the nose, and following the lines of the preceding argument an attempt was made to do this by decreasing the nasal flora.

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(C). THE TREATMENT OF CARRIERS BY MEANS OF VACCINES.

The first means adopted to influence the flora of the nose was the administration of vaccines.

Two series of cases were treated in this manner. The procedure was the same in every case, and was as follows:- Swabs were taken from the noses of the carriers to be investigated, and the material so obtained was inoculated on blood plates. This was repeated on several occasions until some idea had been obtained of the normal nose flora of each case. When this has been ascertained, vaccines were manufactured from the organisms isolated. It must be clearly understood that in most cases the aim of the vaccine was not to attack the Klebs-Loeffler bacillus directly, but to influence the organisms which were present along with the K.L.B.

45 vaccines we made in all. These vaccines were autogenous in every case. The great majority of the vaccines was made entirely by myself, and those which were not, were made under my direction.

RESULTS OF THE FIRST SERIES OF CASES.

The following organisms constituted the vaccines used in the first series of cases treated.

Beta haemolytic streptococcus	...	16
Klebs-Loeffler bacillus	...	6
Pneumococcus	...	1
Klebs-Loeffler bacillus and pneumococcus	...	1
Klebs-Loeffler bacillus and Beta haemolytic streptococcus	...	2
Staphylococcus	...	19
(Staphylococcus aureus 7		
Staphylococcus albus. 6		
Staphylococcus albus.. and aureus 5		
Staphylococcus aureus and streptococcus .. 1		
19)		
Total		45.

38 cases were treated in this series. In addition, many more vaccines were made, but these were never used as the cases cleared up without the use of such artificial aid. 32 cases had one vaccine, 5 cases had two vaccines, and 1 case had three vaccines administered.

The dosage of the vaccines was as follows:-

The staphylococcus vaccines contained about 1,500 to 1,800 million organisms per c.c., the staphylococcus aureus vaccines being somewhat more concentrated than those made from the staphylococcus albus. The pneumococcus vaccines contained about 700 million organisms per c.c., while the haemolytic streptococcus vaccines contained about 300 to 400 million

organisms per c.c. The vaccines made from the Klebs-Loeffler bacillus were somewhat stronger, containing 3,000 to 4,000 million organisms per c.c. The micrococcus catarrhalis vaccines contained about 1,200 million organisms per c.c., and the non-haemolytic streptococcus vaccines contained about 200 to 300 million organisms per c.c. The actual dose administered commenced with 0.1 c.c. and was increased at bi-weekly intervals to 0.25 c.c., 0.5 c.c., 0.75 c.c., and then to 1 c.c. This last dose was repeated about six times, after which the vaccine was discontinued.

The cultures taken from the cases treated were all strongly positive, and all the cases were persistent carriers, except one who was an intermittent carrier. In all but one of the 38 cases the physical examination of the nose revealed the presence of considerable abnormality. Most of the cases showed the presence of much crusting in the nose with muco-pus in the naso-pharynx. The virulence of the organisms carried was established in all cases in both series except one. In this case the organism could not be isolated. The ages of the patients treated in both series ranged from nine months to sixteen years.

The results were moderately successful.

(1). <u>In 16 cases a cure was effected after the use of 1 vaccine</u>.

The following table shows the periods after which the cases cleared up:-

Sixteen cases successful.

5	cases	cleared	up	after	0	positive	culture
3	"	"	"	"	1	"	"
1	case	"	"	"	2	"	cultures
1	"	"	"	"	3	"	"
2	cases	"	"	"	4	"	"
2	"	"	"	"	5	"	"
1	case	"	"	"	6	"	"
1	"	"	"	"	9	"	"

The graphs appended at the end of the thesis show the effect of some of the vaccines used. In the successful cases the vaccines used were as follows:-

Staphylococcus vaccine	...	9 cases,
Klebs-Loeffler bacillus vaccine		3 cases,
Klebs-Loeffler bacillus and pneumococcus vaccine	...	1 case,
Beta haemolytic streptococcus vaccine	...	3 cases.

(2). In 22 cases the vaccine produced no effect upon the carrier condition.

7 of these unsuccessful cases cleared up after nasal douching; 1 case cleared up after tonsillectomy; 1 case had

a cleft palate; 3 cases showed K.L.B. present in ear discharge; 5 cases failed to clear up after additional nasal douching without any apparent reason. Nothing of note was found in the remaining 5 cases.

Swabs were taken at weekly intervals from the noses of the carriers under treatment, and poured blood plates were inoculated with the material so obtained. It was found that the organisms for which the vaccines had been administered were in most cases being eliminated from the noses during the treatment. Thus, the vaccines appeared to be efficient. This, however, was not always the cases with the staphylococcus vaccines. In such cases the staphylococcus(both albus and aureus) failed to disappear, and persisted present in all the blood plates made during treatment. The general condition of the noses of carriers under treatment with vaccines improved but not to any very marked extent. Nasal discharge, where such was present, decreased in amount, and crusting of the nose diminished. The condition of the nose, however, never attained an absolutely satisfactory state.

RESULTS OF THE SECOND SERIES.

The vaccines used in the treatment of the first series of cases were composed of the predominant organism or organisms found along with the K.L.B. in the noses of the carriers to be treated. At the suggestion of the Scientific Advisory Committee,

a further series of strongly positive "nose carriers" was selected, and vaccines were made from all the organisms (with the exception of the K.L.B.) found after careful examination of the nose flora. It was proposed to administer these vaccines, and to check the results by the examination of blood plates, in the manner already described. This series of cases is not yet completed, but the results obtained so far will be given. It is also proposed to administer more concentrated vaccines composed of the organisms still present in the nose to those cases which have failed to clear up after a course of the first vaccine.

I also decided to wash out the noses of the carriers under treatment, daily, with a simple alkaline douche. This douching was to be stopped after three weekly negative cultures had been obtained.

The following vaccines were used in the second series of cases:-

Staphylococcus aureus	...	2
Staphylococcus aureus and albus	...	1
Staphylococcus and non-haemolytic streptococcus	...	4
Staphylococcus aureus and micrococcus catarrhalis	...	2
Staphylococcus aureus and Beta haemolytic streptococcus		1
Beta haemolytic streptococcus		1

10 cases in all were treated in this series. All were persistent and very stubborn "nose carriers". 8 had failed to clear up after the removal of tonsils and adenoids, and 2 had failed to clear up after having been given a previous vaccine.

The results of treatment were as follows:-

(1). 3 cases cleared up after treatment. 1 case cleared up as soon as the treatment commenced, and the 2 remaining cases cleared up within 3 weeks.

The vaccines used in the successful cases were composed of staphylococcus aureus in 2 cases, and Beta haemolytic streptococcus in the remaining case. The staphylococcus was not entirely banished from the nose during treatment, but the Beta haemolytic streptococcus did completely disappear.

(2). 3 cases failed to clear up after treatment. 1 case, however, was definitely improved.

(3). In 3 cases the original virulent K.L.B. was found to have become non-virulent, and the cases were subsequently discharged. The virulence tests were repeated several times in two of the cases. This metamorphosis of virulent to non-virulent bacilli is somewhat astonishing, but it must be stated that in two of the three cases non-virulent bacilli had previously been isolated. It would appear then that the virulent bacilli disappeared during treatment, leaving only the non-virulent variety. These were the only occasions on which such an

occurrence took place during the investigation.

(4). The remaining case was given two vaccines in succession. The first vaccine, composed of staphylococcus and micrococcus catarrhalis, had no effect. The second vaccine which was composed of staphylococcus aureus and streptococcus was then given in conjunction with nasal douching and effected an immediate cure.

CONCLUSIONS.

It will thus be seen from the results of the vaccine administration that this form of treatment, even when combined with nasal douching is by no means an unqualified success. The great majority of cases treated, and, indeed the great majority of all the carriers which were included in the investigation, showed the presence of the staphylococcus in the nose. No vaccine used, however concentrated, appeared to be entirely efficient in getting rid of this organism. The failure of the staphylococcus vaccines in the treatment of carriers is, therefore, not surprising. The number of cases where pathological conditions such as crusts, nasal discharge, muco-pus in the naso-pharynx, etc., are caused or kept up by organisms other than the staphylococcus is comparatively small. Such cases appeared quite amenable to treatment by means of the corresponding vaccines. The large majority of cases, however, are as we have seen, outside this category and some other form of treatment was

sought for, which might be efficient in these cases. To meet this difficulty, it was decided to try the effect upon selected carriers of alkaline nasal douching.

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(D). THE TREATMENT OF CARRIERS BY MEANS OF ALKALINE NASAL DOUCHING.

63 cases in all were chosen for treatment by means of alkaline nasal douching. As already stated, the object of this treatment was to render the noses as clean as possible, and to see the effect so produced upon the Klebs-Loeffler bacillus. The douching was not carried out with any idea of eliminating the K.L.B. directly, though, of course, this might be expected to occur during treatment. Indeed, this direct elimination did occur and had to be guarded against when estimating the effect of the treatment. Nasal douching had been carried out in a modified form at the North-Eastern Hospital some time previous to the present investigation, and had not been a great success. It was decided, however, that this time the treatment should have a prolonged trial under very careful supervision. The cases chosen for treatment were all carriers with strongly positive nose cultures. Clinical examination of the noses showed, in practically all cases, the presence of some pathological abnormality, most often crusts or discharge. Most of the cases were necessarily very young children. This increased the difficulties met with in carrying out the treatment. The youngest child treated was only eight months old, while the oldest case was a girl of eighteen years. The method of carrying out the treatment was as follows:-

An alkaline solution was prepared. This solution was recommended by Mr. Marriner, who had already used it as a nasal douche at St. Thomas's Hospital. It was made up of equal parts of sodium bicarbonate, sodium biborate and sodium chloride. Two drams of this mixture were added to one pint of water and kept as stock. When used as a douche this solution was diluted with equal parts of warm water. The only modification in this prescription was the addition of two ounces of hydrogen peroxide to one pint of the solution when the noses to be treated contained many crusts. The effect of the hydrogen peroxide was to soften the crusts and thus aid in their elimination. The solution was run into the nose from a container by means of rubber tubing and a small nozzle which fitted into the anterior nares. The fact that most of the cases treated were very young childrenn with small nostrils made the selection of a suitable nozzle somewhat difficult. The type which was found most satisfactory was a long slender nozzle which had originally been designed for throat irrigation. The noses were douched twice daily, morning and evening, and half a pint of the solution was used on each occasion. The sisters in charge of the wards where treatment was being carried out were given careful instructions as to how to carry out the treatment, and were warned to make sure that the solution, after being passed into the nose, should pass freely out of the

child's mouth.

The nose flora of the cases selected for treatment was examined thoroughly before treatment commenced. Blood plates were made at weekly intervals while the treatment was being carried on, and also until the patient was discharged. In all cases the flora of the nose was extensive and the staphylococcus (albus and aureus) was invariably present. After treatment was commenced the change was startling. Noses which had shown profuse discharge and which had yielded a very extensive flora became practically sterile after a week of douching. This happened in practically every case. Indeed, where such was not the case and when the blood plates made from the nose continued to show the presence of many organisms it was at once suspected that the treatment was not being efficiently carried out. This was, in fact, proved on various occasions. Thus, in several cases, when the douching had failed after a fair trial, an interval was allowed to elapse and the treatment was recommenced under more careful supervision. As will be seen later, cures were obtained in several cases after this second course of treatment. Nevertheless, some cases did not show a diminution in the nose flora after douching even when the method of carrying out the treatment was carefully watched and appeared to be above reproach. The presence of some definite pathological abnormality in the structure of the nose or naso-pharynx was

looked for in these cases. The existence of a large pad of adenoids appeared to be the cause of failure in two cases, and an infection of the antrum of Highmore in one other case. Suitable operative measures put these defects right, and effected a cure in each instance.

In addition, clinical examination was made of the noses at intervals during the treatment, and the improvement seen here was also very marked. The nasal mucous membrane which, before treatment, had shown the presence of large crusts, became pink and healthy. Nasal discharge diminished or ceased altogether. This happened in almost all the cases examined, and was even noted in cases which had shown profuse nasal discharge and crusting for as long as twelve months previously.

The blood plates made after the treatment had been discontinued, however, showed that this sterile condition of the nose did not persist. The flora of the nose usually reappeared as abundantly as before, although the physical condition of the nose had improved. This was only what one had expected. The important point, however, was that, although the normal nose flora reappeared, the K.L.B., where such had been got rid of by the treatment, remained absent, and most of the cases which had become negative during treatment remained so until discharge. This was not always the case, but here the possibility of re-infection became a factor of importance. It is only

reasonable to suppose that the nasal mucous membrane of carriers, especially carriers of six or more months' duration, becomes specially adapted to the growth of the K.L.B. It seems, therefore, that re-infection would be not only possible, but very probable in such cases. It was very difficult to guard against this possibility. The carriers under consideration were often coming in contact with convalescent diphtheria cases before these cases became "negative", and also with other "positive" carriers. The chances, therefore, of re-infection were great no matter what precautions were taken to guard against them. As a matter of fact, all possible precautions were taken in the attempt to segregate the "negative" carriers from "positive" contacts.

As already stated, the cases selected for treatment were specially chosen because of their chronicity and because of the extent to which K.L.B. were being carried in the nose. In many cases other forms of treatment had been tried previously and had completely failed. This especially applies to treatment by means of vaccines, for of the cases treated by nasal douching 14 had been given vaccines without any benefit. Of course, the possibility that the carriers who were being treated would have cleared up without any artificial aid always exists, and the fact that there is a natural tendency for each carrier to clear up, supports this hypothesis. However, as I have shown, the

cases were specially chosen so that the possibility of this happening would be slight. The fact, also, that each carrier had persistent, strongly positive cultures for at least twelve weeks, and often for much longer, before treatment had commenced; and the knowledge that other forms of treatment had failed in these cases, render the possibility of their being in the process of clearing up when treatment was commenced very small. Indeed, none of these cases at the commencement of treatment showed the slightest sign of clearing up.

It was not possible to obtain a series of control cases who would remain untreated while a similar series of cases were having the possible benefit of treatment, as the effect upon the children of a prolonged stay in hospital, and the effect upon the parents if this procedure were known, quite outweighed any benefit which might have accrued from such an arrangement. Moreover, only the most persistent cases were chosen for treatment, carriers in which time alone had had no effect. I therefore considered that it was quite justifiable under the circumstances to regard the period of 12 weeks or more during which the carriers had had no treatment, and during which they had failed to clear up, as constituting a control in each case.

If the nasal douching was carried out in the manner already stated, the cultures almost invariably became negative. There was no guarantee, however, that, once the nasal douching stopped,

the cultures would remain negative. Therefore a period had to elapse between the cessation of the treatment and the discharge of the patient. After consideration I came to the conclusion that the best plan would be to stop the treatment after three negative cultures had been obtained, and to discharge the patient after a further three negative cultures. That is, three weeks interval would elapse between the cessation of treatment and the discharge of the patient. This period appeared quite sufficient to justify the carrier being considered "free from infection", though, of course, it would not eliminate the possibility of re-infection, which had to be guarded against. As a matter of fact, in many cases a much longer interval than three weeks was allowed to elapse between the cessation of treatment and the discharge of the patient and in no case was the three weeks interval encroached upon.

RESULTS OF NASAL DOUCHING.

The complete results of the treatment are as follows:-

(1). No. of cases treated .. .. 62.

Of these cases, treatment had to be stopped after a short time in 7 cases. The reasons were as follows:-

In 1 case the child vomited whenever treatment was carried out.

In 2 cases the child became cyanosed.

In 2 cases the child's general condition was severely affected by treatment.

In 1 case the child was on a spinal frame and treatment could not be properly carried out.

In 1 case the child died of intercurrent broncho-pneumonia following upon heart disease a few weeks after the completion of nasal douching.

(2). No. of cases cured .. .. .. 27.

11 cases became negative as soon as douching was commenced.

7 cases became negative after 1 week.

5 cases became negative after 2 weeks.

2 cases became negative after 4 weeks.

1 case became negative after 6 weeks.

1 case became negative after 7 weeks.

8 of these cases had previously failed to clear up after the administration of a vaccine, and 1 also after the removal of tonsils and adenoids. All the cases had strongly positive cultures

16 were persistent carriers, while 11 were intermittent.

1 case had ear discharge.

(3). No. of cases cured after one relapse .. 8.

3 relapses occurred after 3 weekly negative cultures.

4 relapses occurred after 5 weekly negative cultures.

1 relapse occurred after 8 weekly negative cultures.

7 of these cases cleared up after 1 further positive culture, while the remaining case cleared up after 2 further positive cultures. 6 of the cases were persistent and 2 were intermittent

carriers. 2 cases had ear discharge.

(4). No. of cases which failed to clear up after the first course of treatment, but which cleared up after a second course of treatment 8

The details were as follows:-

Result of first course of treatment.	Result of second course of treatment.
1. Treatment stopped as child refractory.	Case became negative on treatment; child's behaviour improved.
2. No effect produced on cultures; child refractory.	Case became negative on treatment; child's behaviour improved.
3. No effect produced on cultures.	Case became negative after antra of Highmore had been cleaned out.
4. No effect produced on cultures; child refractory.	Case became negative after five weeks' treatment. Child more used to treatment during second course and behaviour correspondingly improved.
5. No effect produced on cultures; child refractory.	Case became negative after three weeks' treatment. Child more used to treatment during second course and behaviour correspondingly improved.
6. No effect produced on cultures; child refractory.	Case became negative after three weeks' treatment. Child more used to treatment during second course and behaviour correspondingly improved.
7. No effect produced on cultures.	Case became negative after eight weeks' treatment.

8. Treatment stopped as child was on splint and treatment was very difficult to carry out. Treatment persisted with, and child became negative after one weeks treatment.

2 of these cases had failed to clear up after the administration of a vaccine, and 1 also after the removal of tonsils and adenoids. 7 were persistent carriers and 1 was intermittent. 2 cases had ear discharge.

(5). Number of cases in which treatment produced no effect .. 12

7 of these cases had 1 course of treatment.

5 of these cases had 2 courses of treatment.

In 2 of these cases ear discharge was present. Both of these cases had failed to clear up after the administration of a vaccine and one also after the removal of tonsils and adenoids.

2 cases were cured after the removal of tonsils and adenoids.

2 cases were cured after the administration of a vaccine. In both of these cases organisms other than the staphylococcus were included in the vaccine, and these organisms seemed to be the prime cause at work in keeping up the abnormal condition of the nose.

3 cases were given vaccines without success.

1 case failed to clear up after the removal of tonsils and adenoids.

1 case failed to clear up after a vaccine had been administered, but in this case the child had a cleft palate.

11 of the cases were persistent carriers, and the remaining 1 was intermittent.

The result of the physical examination of the nose in these failures is very interesting and serves to show that the presence of pathological abnormalities played a large part in the production of these failures.

The results of the examination of the nose in the cases which failed to clear up after 1 course of treatment were as follows:-

(1). "Ozoena present" (ear discharge also present).

(2). "Nose fairly clear".

(3). "Nose very dirty".

(4). "Crusts in nose".

(5). "Slight crusts in nose".

(6). "Very slight crusts in nose".

(7). "Crusts in nose".

The results of the examination of the nose in the cases which failed to clear up after 2 courses of treatment were as follows:-

(1). "Crusts in nose, which is very dirty".

(2). "Crusts in nose; muco-pus in naso-pharynx".

(3). "Crusts in nose".

(4). "Crusts in nose".

(5). "Cleft palate (unfinished operation) and very dirty nose".

All the cases treated were virulent except 4 which became negative before a culture could be obtained for testing purposes.

The complete results are, therefore, as follows:-

Total number of cases treated	62		
Number of cases in which treatment had to be stopped	7		
Actual number of cases treated	55		
			per cent
Number of cases cured after one course of treatment..	27	=	49
Number of cases cured after two courses of treatment.	8	=	14.5
Number of cases cured after one relapse	8	=	14.5
Total number of cures	43	=	78
Total number of failures	12	=	22.

The following tables show typical results obtained in the treatment of carriers by nasal douching:-

(1). Case No.206.	No.of colonies on blood plates.	Result of culture, as regards K.L.B.
Average before treatment began	++++	+++
1st.week of nasal douching	+ a few	-
2nd. week of nasal douching.	+	-
3rd week of nasal dpuching	++++	++

Case No.206.	No.of colonies on blood plates	Result of culture as regards K.L.B.
4th week of nasal douching	++	-

Nasal douching discontinued after two more negative cultures.

1st week after nasal douching was discontinued	+++++	-
2nd week after nasal douching was discontinued	+++++	-
3rd week after nasal douching was discontinued	+++	-

Case ready for discharge.

Note the reappearance of colonies on the blood plates after the cessation of the nasal douching, without the reappearance of K.L.B. in the cultures.

* * * * *

(2).Case No.205.	No.of colonies on blood plates	Result of culture as regards K.L.B.
Average before treatment began	+++++	+++
1st week of nasal douching	+	+
2nd week of nasal douching	++	-
3rd week of nasal douching	++	++
4th week of nasal douching	+	-

Nasal douching discontinued after two more negative cultures.

1st week after nasal douching was discontinued	++	-
2nd week after nasal douching was discontinued	++	-
3rd week after nasal douching was discontinued	Practically sterile	

Case ready for discharge.

(3). Case No.208	No.of colonies on blood plates	Result of culture as regards K.L.B.
Average before treatment began	+++++	++
1st week of nasal douching	++++	+
2nd week of nasal douching	Sterile	+
3rd week of nasal douching	+ few	-
4th week of nasal douching	Practically sterile.	-
Nasal douching discontinued after two more negative cultures.		
1st week after nasal douching was discontinued	+	-
2nd week after nasal douching was discontinued	++	-
3rd week after nasal douching was discontinued	+++	-
Case ready for discharge.		

* * * * *

(4). Case No.198	No.of colonies on blood plates	Result of culture as regards K.L.B.
Average before treatment began	+++++	++
1st week of nasal douching	+++	++
2nd week of nasal douching	++++	-

Case No.198	No.of colonies on blood plates	Result of culture as regards K.L.B.
3rd week of nasal douching	++++	-
4th week of nasal douching	+++	-
Nasal douching discontinued after two more negative cultures.		
1st week after nasal douching was discontinued	+++	-
2nd week after nasal douching was discontinued	+++	+

Note the difficulty in removing organisms from the nose, with the reappearance of K.L.B. in the cultures. The child was very nervous. This case was eventually cured by the removal of the tonsils and adenoids.

When carriers who had cleared up after a course of nasal douching were discharged from hospital, the parents were interviewed and asked to bring the children up for examination after four weeks. Only three parents complied with this request. Swabs were taken from the noses and throats of the children who arrived, and in all three cases the results were negative.

CONCLUSIONS.

It will be seen that the results of nasal douching were quite satisfactory. Success was obtained quickly in quite a large proportion of cases, and where the cases did not clear up immediately a further course of treatment often produced the desired effect. The cases which did not clear up were

all very strongly positive and persistent carriers, and the explanation of the failure could be found in the presence of marked abnormalities in the nose. The failure of nasal douching in those cases was to be expected, but the fact that the cases which did clear up practically always showed the presence of abnormalities in the nose demonstrates that this is not an insurmountable difficulty.

There is no doubt that nasal douching remains by far the most hopeful and most logical form of treatment for "nose carriers" tried during the investigation.

The main difficulty met with lies in the fact that, although nasal douching is quite painless, some children become afraid at sight of the douching apparatus, and struggle a good deal, thus impairing the efficiency of the treatment. Steps are now being taken, however, at the North-Eastern Hospital to devise some modification in the form of a spray whereby the refractory child can be dealt with more easily, as it is felt that success is almost assured if the confidence of the child can be gained.

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(E). THE TREATMENT OF CARRIERS BY MEANS OF THE REMOVAL OF TONSILS AND ADENOIDS.

As a corollary to the treatment of carriers by means of vaccines and nasal douching it was thought that a series of cases should be selected who might benefit by the removal of their tonsils and adenoids.

The literature upon this subject, already referred to earlier in this thesis, makes little, if any distinction between carriers who had K.L.B. present in the nose and throat, and those who had K.L.B. present in the throat only. The series of cases chosen in this investigation, therefore, was specially selected to include these two types of cases. Of course, the presence of K.L.B. in the throat only is not conclusive evidence that there are no K.L.B. in the nose, as the bacilli in the nose may be present only in the region behind the middle turbinates,where it is difficult to obtain material for examination. Nevertheless, if a long series of negative nose cultures is obtained from a case, it is reasonable to assume that there are no K.L.B. present there.

I hoped that by choosing a large enough series of cases, and including in this series (a) carriers with K.L.B. present only in the throat, (b) carriers with K.L.B. present in the throat and occasionally or in small numbers in the nose, and

(c) carriers who in addition to having K.L.B. present in the throat had strongly positive and persistent nose cultures, that some further idea could be obtained as to the relative importance of the "nose carrier" as opposed to the purely "throat carrier".

67 cases in all were treated by the removal of tonsils and adenoids. This total, however, requires some modification before the results can be considered. In the first place, no adenoids were obtained from 4 cases. As, however, the site of adenoid growth was scraped in each of these cases, this fact did not appear of any great importance. In addition, 5 cases had several negative cultures before the operation was performed. In each of these cases no positive cultures were obtained after the operation, but it was not considered desirable to include them among the list of cases treated. Lastly, 2 cases were discovered to be carrying non-virulent organisms, and were also omitted from the final total.

The total number of cases which I will consider is, therefore, 60. 34 of these cases were boys under fifteen years of age, and 19 were girls under fifteen years. The remaining 7 were adults. Of these adults 5 were nurses, the remaining 3 being males. The ages of all the cases treated by operation varied from 1 to 27 years.

The operations with one exception were done in the theatre

of the North-Eastern Hospital by Mr. Marriner of St. Thomas's Hospital. The remaining case was operated upon by Mr. T.B. Layton of Guy's Hospital.

Before a case could be discharged following upon the operation, it was necessary to obtain 6 negative cultures taken at weekly intervals. In addition, I never took a culture during the week following upon the operation as the condition of the throat made the taking of the culture very uncomfortable to the patient, while the number of pyogenic organisms present in the throat at that time rendered the likelihood of obtaining any K.L.B. rather remote.

RESULTS OF TREATMENT.

The complete results of this series of cases are as follows:-

(1). Number of cases included in the series 60

(2). Number of cases cured by the removal of tonsils and adenoids ... ... 35.

Number of immediate cures following the operation ..25

Of these cases, 12 had bacilli in the nose and throat, while 13 had bacilli in the throat only.

Number of cases cured after having had 1 positive weekly culture following upon the operation 5

Of these cases, 2 had bacilli in the nose and throat, 2 had bacilli in the throat only, and 1 had bacilli in the throat and left ear.

Number of cases cured after having had 2 positive weekly cultures following upon the operation 2

1 case had bacilli in the nose and throat, and 1 had bacilli in the throat only.

Number of cases cured after having had 3 positive weekly cultures following upnn the operation 1

This case had bacilli in the nose and throat.

Number of cases cured after having had 4 positive weekly cultures following upon the operation 2

These 2 cases had bacilli in the nose and throat.

It will thus be seen that the cases which took some time to clear up all had K.L.B. present in the nose.

The cases which cleared up when carrying bacilli in the nose and throat did not show strongly positive nose cultures. Where the nose cultures were strongly positive the operation was not successful.

Among the 7 adults treated by operation, 5 cleared up immediately. 1 cleared up after having had 1 positive culture, and the remaining case cleared up after 4 weekly positive cultures following upon the operation.

4 nurses not included in this series had their tonsils and adenoids removed while carrying K.L.B., but before the twelve

weeks had elapsed which would have made them officially "chronic carriers". The operation was immediately successful in each of these cases, all 4 clearing up without any further positive cultures.

(3). Number of cases which failed to clear up after the removal of tonsils and adenoids 25

Of these failures 24 carried K.L.B. in the nose and throat. The remaining case was purely a "throat carrier", and this case relapsed after having had 5 negative cultures following upon the operation. After this 1 positive culture, the remainder were all negative again, and the case was discharged after the customary six weeks. The reason for this relapse could not be found as there did not appear to be anything abnormal about the throat, and the operation had been quite successfully performed.

7 of the failures had ear discharge.

2 of these had double otorrhoea,

1 had left otorrhoea,

4 had right otorrhoea.

Of the remaining failures:-

2 cases were cured after the administration of vaccines.

2 others were cured after nasal douching.

Another case cleared up after having the antra of Highmore opened and cleaned.

8 other cases failed to clear up after having either vaccines or nasal douches.

The remaining 5 cases had no further treatment, and eventually cleared up after a prolonged period.

The following table gives the percentage of cures in the cases which had bacilli in the nose and throat, and once more shows the influence of a positive nose culture upon the success of the operation.

Of the 60 cases which had their tonsils and adenoids removed,

16 had bacilli in the throat only.
15 of these cases were cured ... = 94 per cent cured
(The only failure was the "relapse" already noted).

36 had bacilli in the nose and throat.
18 of these cases were cured ... = 50 per cent cured

7 cases had ear discharge. Of these only 1 cleared up ... = 14 per cent cured.
(The only case among these 7 who cleared up was one who showed K.L.B. in the throat and ear only. The remaining cases had bacilli in the nose, throat and ear).

THE REMOVAL OF ADENOIDS.

16 of the cases operated upon were chosen for operation principally to see the effect upon the carrier state of the removal of adenoids. In no case, however, were the adenoids removed without the tonsils, as the latter always showed sufficient evidence of abnormality or definite disease to

warrant their removal. All the 16 cases chosen had strongly positive nose cultures. 3 out of the 16 cases were cured, and K.L.B. were isolated from the adenoids in 2 of these cases. Altogether in the series of 16 cases K.L.B. were isolated on 6 occasions from the adenoids obtained. The importance of this fact will be seen when it is realised that in the remaining 44 cases where the operation was not performed primarily to remove the adenoids, K.L.B. were isolated from the adenoids in only 8 cases.

From this it will be seen that the removal of adenoids is not highly successful in the cure of nasal carriers.

The foregoing tables and data show the importance of the "nose carrier". From the work done it was quite clear that when the nasal cultures were positive a cure was less likely to be obtained by the removal of the tonsils and adenoids than in a case where the throat cultures only were positive. Where the nose cultures were strongly and persistently positive, the likelihood of a cure resulting from the operation was decidedly remote.

THE EXAMINATION OF THE MATERIAL OBTAINED AT OPERATION.

The tonsils and adenoids obtained at the operation were immediately removed to the laboratory, and an attempt was made to isolate K.L.B. from them. This was done in the case of the tonsils by sectioning the specimens and obtaining material

from the crypts. A platinum wire was used to penetrate the crypts, and the material so obtained was spread on tellurite-serum-agar plates. It was my custom to make two plates from each tonsil obtained. The adenoids were treated in much the same way, material being expressed from the specimens and inoculated on to the same type of plate. The plates so made were incubated for twenty-four hours at 37°C. and then examined. If no growth resulted, a further twenty-four hours' incubation was resorted to, and the plates were again examined under the low-power binocular microscope.

Suspicious colonies were picked off and examined microscopically. K.L.B. were isolated, where present, and if the virulence of the organisms had not already been established, this was done. As a matter of fact, virulence tests were done upon practically all the K.L.B. isolated from the tonsils at operation, irrespective of whether or not the virulence had already been established. It is interesting to note that 9 cases had negative throat cultures before the operation and still showed K.L.B. present in the crypts of the tonsils removed at the operation. The examination of the tonsils revealed the fact that the type which was most likely to carry K.L.B. was not the very large tonsil, but that one which, no matter what its size might be, <u>showed the presence of large and deep crypts</u>.

The result of this part of the work is as follows:-

K.L.B. were isolated from the tonsils and adenoids in 13 cases.
K.L.B. were isolated from the left tonsil only in ... 4 cases.
K.L.B. were isolated from the right tonsil only in .. 8 cases.
K.L.B. were isolated from both tonsils in .... 9 cases.
K.L.B. were isolated from the adenoids in .... 3 cases.
No K.L.B. were isolated from the tonsils or
adenoids in ... ... 23 cases.

Several anomalous results were obtained. In one case non-virulent organisms were isolated from the left tonsil and the adenoids, while virulent organisms were isolated from the right tonsil. In a second case virulent organisms were isolated from the right tonsil and Hofmann's bacillus from the adenoids. Two cases showed the presence of non-virulent organisms in the left tonsil, and virulent organisms in the adenoids, while in one of these cases an organism morphologically resembling K.L.B. but which could not be isolated was found in the right tonsil. In another case non-virulent organisms were isolated from the right tonsil, and organisms which resembled K.LB. but which could not be isolated were found in the adenoids. This case had already been proved to be harbouring virulent K.LB. in the ear.

All the material obtained at operation was fixed in 10 per cent formol-saline, and sectioned. These sections were stained (a) with Haemotoxylin-Eosin, (b) with Giemsa's stain, and (c)

by Twort's method. Microscopical examination of these sections showed the presence of considerable fibrous tissue and inflammatory reaction on the surface of the tonsil. K.LB. were seen lying in the crypts, the latter being numerous and often extending very deeply into the interior of the tonsil.

Of the 56 cases dealt with, the virulence was established in 48 cases. Insufficiently positive cultures were obtained from the remaining 8 to allow of their virulence being established. Several micro-photographs of these sections are shown at the end of the thesis.

CONCLUSIONS.

The final conclusion which I drew from a consideration of the results obtained from these operations was that the removal of the tonsils and adenoids is of the greatest value where the carrier has bacilli in the throat only. The likelihood of a cure becomes less in proportion to the extent to which K.L.B. are present in the nose. In a strongly positive "nose carrier" the operation is practically useless.

It was not possible in this investigation to obtain a large enough series of carriers of less than twelve weeks' duration upon which the operation could be performed. After consideration of the results obtained from all the cases treated, including the four cases which were operated upon before they officially

became "carriers", I think it quite reasonable to assume, however, that, if the operation were carried out extensively in carriers of less than twelve weeks duration, the incidence rate of "chronic carriers" would be greatly lessened. In other words, early removal of tonsils and adenoids in suitable cases would considerably lessen the duration of the carrier condition. The examination of convalescent diphtheria cases four weeks after admission to hospital showed that quite a large percentage of these cases have lesions in the tonsils. There would, therefore, appear to be a certain percentage of cases at that time in which the removal of the tonsils and adenoids was justifiable. It is difficult to state what period should be allowed to elapse after the initial attack of diphtheria before the operation becomes advisable. If the time at which the patient is out of danger, and at which the likelihood of complications arising is considered, and if the loss of time and education to the patients, who are mostly children, together with the economic burden which they lay upon the community is also taken into account, it would certainly seem wise to carry out this operation considerably earlier than has hitherto been the case. I think the optimum time for operation would probably be six to eight weeks after admission to hospital.

This argument for the early treatment of diphtheria carriers with a view to lessening the duration of the carrier

state is one of great importance, and applies not only to the removal of tonsils and adenoids, but to every preventive and curative measure which might be tried. A fuller discussion of this point is left until later in the thesis.

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(F). ADDITIONAL FORMS OF TREATMENT.

(1). The use of iodine oil.

The use of antiseptics applied to the nasal mucous membrane had been tried in the North-Eastern Hospital at various times before I commenced this investigation. The results obtained by this form of treatment were unsatisfactory, and as the methods adopted to carry out the treatment appeared to have been excellent, it did not seem sufficiently worth while to spend much time in repeating this work. The main findings obtained by those working in the North-Eastern Hospital were that the use of antiseptics, especially iodine oil, eliminated the K.L.B. entirely during the course of treatment, but that when the treatment was stopped the K.L.B. reappeared. Various forms of antiseptics had been used previously, such as iodine oil, iodine and iodex, with much the same result.

I applied a one per cent solution of iodine oil to the noses of four children. Three of these cases cleared up after treatment and one case did not clear up. None of the cases, however, were very strongly positive or persistent "nose carriers", and there was some evidence that the cultures were becoming negative before treatment was commenced. In view of the previous work done in the hospital I did not pursue this line

of investigation further.

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(2). Antrum investigation.

When it was seen that the cultures of several "nose carriers" remained strongly positive after the administration of vaccines and nasal douches, even when the physical condition of the nose improved and nasal discharge and crusting disappeared, it was thought that possibly the antrum of Highmore might be serving as a nidus for the growth of the K.L.B. It was, therefore, decided to select several carriers, and to have the antra explored with a view to discovering what condition prevailed within, and to see if it were possible to isolate K.L.B. from the sinus.

To further this object, X-ray photographs were taken of all the carriers whose cultures remained positive after treatment, and also, for comparison, of several whose cultures had cleared up on treatment. It was hoped in this way to obtain some assistance in forming an opinion as to the condition of the antrum. At least 50 cases were X-rayed for this purpose. The results, however, were very unsatisfactory. The children were very young, and the process of being X-rayed appeared very terrifying to them. The result was that the children moved, and the photographs obtained were of very little use. A general

anaesthetic would have been necessary to obtain good photographs, but this procedure was not considered justifiable under the circumstances.

Three cases had their antra of Highmore explored by Mr. Marriner. These three cases had all failed to clear up after nasal douching and vaccines. In addition, one of the cases had failed to clear up after the removal of tonsils and adenoids. In all these cases the antra were opened from the nose, a cannula being inserted and saline run into the cavity. Before this was done a strip of gauze soaked in strong iodine solution was inserted in each nostril and left in for one minute, thus rendering the possibility of contamination of the fluid or cannula practically negligible. All other precautions as to the sterility of the fluid for examination were attended to carefully. In two out of the three cases so examined K.L.B. were found in the fluid obtained from the left antrum. In the third case the fluid obtained showed no K.L.B., but the cannula became blocked in one antrum and the amount of fluid obtained back was quite insufficient for a proper examination.

One of the cases in which K.LB. had been found in the antrum, and the case in which no K.L.B. had been found, cleared up after having had a second course of nasal douching following upon the operation. The third case, in which K.L.B. had been

found in the left antrum, failed to clear up after nasal douching, and so it was decided to open the left antrum and clean it out thoroughly, at the same time removing the tonsils and adenoids. This was done and the case cleared up without any further positive cultures. No K.L.B. were found in the tonsils or adenoids at operation. In this case it is interesting to note that the left nostril consistently showed much more discharge than did the right nostril. I could not get any further cases to include in this series, as it was not considered desirable to operate upon very young children where the antra would not be fully developed. In addition, it was sometimes difficult to get the necessary permission to carry out the operation. It appears, however, that this point is of some importance. The antrum of Highmore is an ideal position in which K.L.B. can multiply and be discharged into the nose. No ordinary form of treatment, short of radical operation, will avail in these cases. This should always be kept in mind as a last resort.

The ages of the three cases which were treated in this series were 6 years, 6 years and 3 years, respectively.

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(3). Mastoidectomy.

Two carriers were treated by mastoidectomy. Each of these

cases had ear discharge in which K.L.B. were present. The operation of mastoidectomy was performed by Mr. Layton of Guy's Hospital. In one case the operation was successful, and in the other case the cultures became positive again after having been negative for eleven weeks. The number of cases in which mastoidectomy is justifiable appears to be quite small. In each of the two cases just referred to, the operation was done with a view to stopping the ear discharge and not with the idea of bringing the carrier condition to an end. I have included these cases, however, to show that a cure can result from this operation.

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(4). Hygienic Measures.

The general health of the carriers included in this investigation received careful attention. An investigation of all the carriers showed, however, that the general health had apparently little, if anything, to do with the carrier condition. As a matter of fact, the great majority of carriers, after the initial attack of diphtheria had been recovered from, remained extremely healthy throughout their stay in hospital. In addition, the severity of the initial attack of diphtheria did not appear to have anything whatever to do with the subsequent onset of the carrier condition. That is, within limits to be

discussed later, a mild attack of diphtheria was just as likely to be followed by a carrier state as a very severe attack. The following table gives some idea of the kind of case with which I had to deal:-

Number of healthy carriers	280
Number of carriers with signs of general debility	44.

The specific affections met with were as follows:-

Tuberculosis of bones or joints.	Congenital Syphilis.	Feeble Mindedness.	Cleft Palate.	Heart Disease.	Impetigo.
6	3	5	2	3	1

Several of the carriers suffered from various exanthemata during their stay in hospital, but this did not affect in any way the duration of the carrier state. One case in particular developed scarlet fever soon after becoming a carrier, and the culturesbecame negative with the onset of this disease. As soon, however, as the throat condition, which had been very septic improved, the cultures once more became positive. One carrier died of broncho-pneumonia, following upon heart disease, just as the cultures were becoming negative. One other interesting case remains to be noted. A child who appeared distinctly feeble-minded and who was a persistent "nose carrier" was put upon nasal douches. The douching caused cyanosis, and soon had to be stopped. The child's general condition, however,

improved enormously, and synchronously with this improvement his cultures became negative and he was soon discharged. This is, however, the only case of its kind which I noticed during the investigation.

On the whole, therefore, the general condition of the carriers was extremely good, and the health of the carrier certainly did not seem to affect in any way the duration of the carrier condition.

The use of artificial sunlight was resorted to in several cases. The cases treated were mostly those with ear discharge and those few who appeared malnourished in any way. The form of light used was that obtained from a carbon arc, and the treatment was properly supervised by an efficient staff. In no case, however, did any benefit result from this form of treatment as far as the carrier condition was concerned, although in many cases the general health of the child was considerably improved.

General heliotherapy was also used whenever the state of the weather permitted. Though this improved the general condition of the carriers, once again no benefit was obtained as regards the carrier condition.

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(5). Isolation.

The question of the isolation of the carriers was not an

easy one. The North-Eastern Hospital chiefly admits acute diphtheria and scarlet fever cases. Several wards were set apart for the diphtheria carriers, but as the number accumulated the accommodation became insufficient, and the carriers overflowed into wards which contained acute diphtheria cases. The carriers were kept separate from all "positive" cases as far as possible, their play-hours in the open and even their time out of bed being, where possible, different from those of all the other patients. In several cases carriers were given separate cubicles or rooms, and kept entirely apart from the other patients. The utensils, feeding and otherwise, of the carriers were, of course, kept strictly separate from those of all the other patients. Even with all these precautions, however, it was not always possible under the circumstances to keep the carriers strictly isolated. The question, therefore, of re-infection of a "negative" case by a new strain of K.L.B. could never be entirely disproved. All precautions, however, were taken to limit this occurrence. In this connection, one interesting case was noted. This was a boy who was put on nasal douching and who yielded fourteen negative cultures at weekly intervals. He was detained in hospital during this fourteen weeks for reasons which I need not enumerate, and had nasal douches at intervals. The fifteenth cultures from

the nose and throat then proved to be positive. This appeared to be undoubtedly a re-infection, as no focus for the growth of K.L.B. could be found. It seems to support the view that the nasal mucous membrane, having harboured K.L.B. for long periods on its surface, can remain sensitive to re-infection for a considerable time. This view received further corroboration during the investigation. Three of the carriers dealt with were discharged from hospital in the usual way after having had six weekly negative cultures carefully examined by myself and, in addition, twelve bi-weekly negative cultures examined synchronously by the medical officer in charge of the ward. These three carriers were later re-admitted to hospital carrying virulent K.L.B. As the cases were undoubtedly"negative" when discharged from hospital this could most probably be explained as a re-infection.

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(G). THE IMMUNITY OF CARRIERS.

A considerable amount of work has been done within recent years upon the presence and amount of diphtheria antitoxin in the blood of carriers. The great bulk of this work has gone to show that antitoxin is definitely present in the blood of all true diphtheria carriers. Loos[89], in 1896, was one of the first to work on this subject, and E. Neisser[90], in 1902, Kleinschmidt[91], in 1913, and Otto[92], in 1914, all found varying amounts of antitoxin in the blood of carriers. The work of Renault and Lévy[93], and Lévy and Léobardy[94], on the other hand, tended to show that antitoxin was not invariably present in the blood of carriers, but the virulence of the organisms which these workers dealt with was never thoroughly established, and so their work has not been generally accepted.

THE SCHICK TEST.

One would expect to find that the immunity of carriers against diphtheria was high, and steps were taken during the investigation to verify this fact. We have in the Schick test a standardised and easily carried out method of estimating the resistance against diphtheria. I carried out the Schick test on all the carriers included in my series, and the test was performed in the accepted manner. The material for the test was obtained from Messrs. Burroughs Wellcome's laboratories.

The test was negative in all cases but two. Of these, one was proved to be carrying non-virulent organisms at the time of the test. Virulent organisms were never found in this case. The second case was more interesting. This was a boy, thirteen years of age, who was carrying virulent bacilli in the nose and throat. He had large tonsils and thin crusts in the nose. The Schick test when done was strongly positive. I immunised the boy with three one c.c. doses of toxin-antitoxin mixture, and I then repeated the Schick test several weeks later. The result was negative. The cultures soon afterwards became negative without any specific treatment having been administered. I have attempted to explain this phenomenon when dealing with the general conclusions arrived at from this part of the work.

In one batch of 15 cases the results of the test were unsatisfactory, the tests and controls being equally positive. In these cases, however, the material used had been kept too long and the results could not be accepted.

Many pseudo-positive and pseudo-negative reactions were obtained but these were neglected.

AGGLUTINATION AND COMPLEMENT FIXATION.

I carried out 5 agglutination tests with the blood of carriers against virulent K.L.B. In 3 cases, the serum of the carriers agglutinated the organisms in a titre of 1/64 after three hours in the water-bath at 55°C., while in the remaining

2 cases no agglutination was obtained. 7 controls were done, using the blood of non-carriers. In each case there was no agglutination.

I tested the complement fixation power of the blood of 3 carriers and 5 controls, using a suspension of K.L.B. as my antigen. In all cases the results were entirely negative.

CONCLUSIONS.

The result of this part of the investigation is apparently to show that diphtheria carriers have a certain immunity against diphtheria. This, of course, is what one would have expected and what has been found almost invariably in the past by competent workers.

The facts that the humoral immunity of carriers is high, and that these carriers have abundant bacilli in the nose and throat for very long periods without any decrease in the number of these bacilli, raise the very important question as to whether the bacilli are living an entirely extra-corporeal existence. The large proportion of cases in which the physical examination of the noses of carriers revealed the presence of crusts, as opposed to the comparatively small number of abnormalities found in the noses of the controls; and the considerable number of cases met with in which K.L.B. when present in the throat were isolated from the secretion of the crypts of the tonsils, supports this theory. In addition, this

theory would explain the cases which have a positive Schick reaction or show the absence of antitoxin in the blood while carrying virulent K.L.B. in the nose or throat. Thus, the solitary case which I obtained of a positive Schick test had considerable fine crusting in the nose, with definitely enlarged tonsils, and would, therefore, support this theory.

A certain degree of immunity must necessarily be present to protect the carriers from the effect of the toxins produced by the bacilli, but if these bacilli are living extra-corporeally, this protection need not be so well developed as it otherwise would require to be.

More work requires to be done on this subject both as regards the antitoxin content of the blood and its variation at different periods during the carrier condition, before this question can be fully dealt with.

Several workers, notably Langer[95], 1916, Durand[96], 1918, Havens[97], 1920, Bell[98], 1922, and Scott[99], 1923, claim to have isolated different serological strains of K.L.B. I obtained the sera corresponding to 9 different serological strains of K.L.B. from Dr.W.M.Scott, of the Ministry of Health, and tested them out against 28 different strains of K.L.B. isolated from carriers. So far I have not obtained any correlation in my results, but I hope to obtain a larger series of cases upon which to work. If it could be proved that there existed a definite type of K.L.B. confined to carriers, our ideas of immunity would have to be considerably altered. -oOo-

(H). THE "EAR CARRIER".

The most difficult carriers met with during the investigation were those who carried virulent K.L.B. in the ear.

Ear discharge during convalescence from diphtheria is not uncommon, and if the discharge persists for any length of time it is very unresponsive to treatment. 39 of the carriers dealt with had organisms resembling K.L.B. in the ear discharge. 36 of these bacilli were proved to be virulent K.L.B., 1 was proved to be non-virulent, and 2 were proved to be "saccharose fermenters". The virulence test in the case of the K.L.B. found in the ear discharge presents more difficulties than does the virulence test of the K.L.B. found in any other region. In the large majority of cases diphtheroids exist along with the K.L.B., and unless several colonies are picked off for isolation and virulence testing, one is liable to obtain only a diphtheroid and not a true diphtheria bacillus. In addition, many of the ear discharges contain a B.proteus-like organism which in culture rapidly overgrows all the other organisms and renders their isolation very difficult. As regards the actual discharge from the ear, many of the carriers showed this discharge without any K.L.B. ever appearing in it. This was due to the fact that in these cases the discharge was post-scarlatinal and not post-diphtheritic. This point has always to be kept in mind.

The treatment of these ear cases was extremely difficult. Indeed, no satisfactory form of treatment was discovered, though many were tried. The discharge would often cease altogether for a period of several weeks and then, without any apparent cause, commence again. The usual methods adopted to treat such discharge were carried out in the wards, the commonest being the instillation into the ear of hydrogen peroxide and alcohol drops. In addition, cases were treated by means of vaccines, removal of tonsils and adenoids and mastoidectomy, while nasal douching was even attempted in some cases. The results of these forms of treatment were as follows:-

	Cures.	No effect.
Removal of tonsils and adenoids ...	3	3
Vaccines (consisting of organisms .. isolated from the nose).	2	1
Nasal douching	3	1.
Mastoidectomy	1	1.

1 case failed to clear up after the removal of tonsils and adenoids, nasal douching, and vaccines.

1 case failed to clear up after the removal of tonsils and adenoids, but cleared up after a vaccine had been administered.

1 case failed to clear up after the removal of tonsils and adenoids, but cleared up after mastoidectomy.

1 case failed to clear up after the removal of tonsils and adenoids, and the administration of iodine oil to the nose.

1 case failed to clear up after mastoidectomy.

It must be remembered that in every case except one (in which mastoidectomy was carried out) K.L.B. were present in the nose or throat as well as in the ear discharge. The treatment was, therefore, directed to removing the bacilli from the nose or throat, these sites being regarded as the sources of infection. It was hoped that if the bacilli were removed from these areas they would eventually disappear also from the ear. The results of the various forms of treatment tried show that this does occur in certain cases.

The stubbornness with which the ear discharge resisted treatment will be seen from the fact that carriers with K.L.B. present in the ear discharge remained "positive" for an average of 29.6 weeks. The average duration of the carrier state in all cases was 24.9 weeks.

Recently ionization has been recommended for the treatment of ear discharge. Proper facilities were not available to carry this out during the present investigation, but it is hoped that these will soon be forthcoming, and that this form of treatment will have a prolonged trial.

Treatment by artificial sunlight was given to several of the carriers with ear discharge, and it was expected that beneficial results would be obtained. This hope, however, did not materialise, the treatment producing little, if any

effect upon the ear discharge.

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THE RESULTS OF THE WORK DONE.

I now propose to consider the results obtained from the present investigation into the diphtheria carrier problem.

In the first place, the work done in making a general survey of the carriers, brought out the importance of the "nose carrier". It was obvious that, if the small number of cases with involvement of the ear were omitted from consideration, the carriers who had been present longest in the hospital were those who carried K.L.B. in the nose. Thus, from an early stage in the investigation the importance of the "nose carrier" was realised.

The work done upon the flora of the throats of carriers and controls yielded information of a purely negative character. From the results obtained it was evident that the carrier state was not dependent upon any specific flora in the throat. The investigation of the flora of the nose was of more positive value. Here again the nasal flora of carriers did not differ qualitatively from that of the controls, but quantitively there was a vast difference. The nasal flora of the carriers was undoubtedly far more abundant than was the case with the controls, and the predominant organism present was the staphylococcus. The nose flora of intermittent carriers was hardly so

extensive as that of persistent carriers, but otherwise little difference was apparent in the two types of cases.

The next point of investigation was to discover firstly, the cause of the abundant flora in the noses of carriers; and secondly, when that was discovered to ascertain what the effect of altering this flora would be upon the carrier state. This brought into prominence the investigation of the pathological conditions present in the noses and throats of carriers, and their identification as possible causes of the abundant flora.

The results of this part of the investigation showed that pathological abnormalities were much commoner in the noses and throats, but especially in the noses, of carriers. In most cases, moreover, these pathological abnormalities were accompanied by a large increase in the normal flora of the region affected, It was very difficult to find any correlation between the intermittency of the carrier condition and the presence of pathological conditions, but cases which were becoming negative undoubtedly had cleaner noses than those who were continuing to have positive cultures. Whether the pathological abnormality preceded or followed the increase in the number of organisms present was a difficult point,but in most cases the abnormal condition present in the nose appeared to be of old standing. At any rate, the physical examination of the noses of convalescent diphtheria patients showed that those cases with pathological abnormalities present

were more likely to become carriers than those whose noses showed no such abnormalities. It did not appear that the carrier state was purely a function of the micro-organism concerned, as the K.L.B. seldom became one of the normal flora of the nose or throat without a pathological process being present. It was not possible to answer one of the questions which followed upon this last point, I hoped to be able to inoculate the throats of cases recovered from diphtheria with virulent K.L.B. isolated from carriers, and thus to see if a carrier state could be induced. Permission to do this was refused. As, however, the K.L.B. did not appear to be able to create a carrier state without artificial aid this point lost much of its significance.

Various means of decreasing the flora of the nose were next tried. For this purpose autogenous vaccines were prepared and administered to a certain number of carriers, and nasal douching was carried out in selected cases. In each case, a proportion of cures resulted. That is, in some cases the flora of the nose was greatly diminished, while at the same time the K.L.B. disappeared. If the nasal douching was carried out properly, the results obtained were much more satisfactory than those procured from the administration of vaccines. In addition, the cost was very slight and the results were quickly obtained. The combination of nasal douching and vaccines as a form of

treatment was also successful. Another recommendation for nasal douching was that the treatment could be commenced early in convalescence. Indeed, all forms of treatment for carriers should be commenced earlier than is at present the case, as the longer the carrier state is allowed to exist the less amenable to treatment does it become.

The results obtained from decreasing the nasal flora were thus very satisfactory, and showed that the best means of attacking the K.L.B. appeared to be through the abundant co-existent organisms. When such methods of treatment failed, a direct attack was made upon the pathological conditions present in the nose. In the few cases treated thus, e.g. by opening and cleaning out the antra, the results were satisfactory.

In the case of the "throat carrier", removal of the tonsils and adenoids was eminently satisfactory. The number of failures increased directly with the extent to which the nose cultures were positive.

The carrier who had K.L.B. present in discharge from the ear was very unresponsive to treatment. In such cases treatment of the nose flora by vaccines and nasal douching, removal of the tonsils and adenoids and mastoidectomy were tried with moderate success.

The investigation of the amount of protective substances in the blood of carriers showed that the carriers had a considerable degree of acquired immunity. This

fact taken in conjunction with the existence of pathological conditions and the beneficial results obtained from the correction of these lead one to the assumption that the K.L.B. was living an extra-corporeal existence. This last hypothesis is one of considerable importance, and its substantiation would serve to explain the large measure of success obtained by the removal of crusts from the nose. In addition, the fact that the crusts, etc. often reappeared in the nose after the cessation of douching, whereas the K.L.B. did not reappear, supports this theory. The reappearance of the K.L.B. in the nose after a few weeks' interval during which negative cultures were obtained could thus be explained as the re-infection of an already sensitive area. As already stated, all precautions were taken to avoid such re-infection, but under the conditions prevailing during the investigation it was quite impossible entirely to preclude such an occurrence.

It was not easy to explain why some diphtheria patients should become carriers, while others did not. The fact that the carriers examined showed a high incidence of pathological abnormalities in the nose and throat; and the fact that all the diphtheria patients examined after four weeks in hospital who became carriers also showed implication of the nose and throat, would appear to prove that the presence of such physical defects in the nose and throat is the deciding factor in inducing the

carrier condition. Nevertheless some cases, when examined after four weeks in hospital and also before discharge, showed the presence of much abnormality in the nose and throat without becoming carriers. Here, however, the virulence of the organism must be taken into account. Many of these cases had only one positive culture or at most only a few positive cultures while in hospital, the initial attack of diphtheria being very mild indeed. As a matter of fact, the only case noted where the nose showed much abnormality on discharge was that of a boy who had had only one positive throat culture during his illness, and that before his admission to hospital. In these cases, therefore, the K.L.B. would never obtain a real hold upon the surface of the mucous membrane. I have already said that the severity of the initial attack of diphtheria appeared to have little, if anything to do with the incidence of the carrier condition. It is interesting to remember, however, that the great majority of the carriers were extremely healthy and well-nourished, and so could have withstood an infection with a virulent strain of organism, which in a less healthy type of child would have produced a severe or even fatal illness. Thus, little information can be obtained from consideration of the severity of the initial attack unless the virulence of the infecting organism and the original resistance of the patient is known. In this connection it would have been

very interesting to note the result of the Schick test on admission to hospital in cases who became carriers. The administration of antitoxin to these patients immediately on admission, however, would have completely negatived the results. We can thus get little information as to the amount of natural antitoxin present in the blood of diphtheria patients who are later to become carriers.

It would appear then that the incidence of the carrier condition is influenced firstly, by the presence of physical abnormalities in the nose; and secondly, by the combination of the virulence of the infecting organism and the resistance of the patient. In other words, a slight infection with B.diphtheriae would not produce a carrier condition unless marked abnormalities were present; while a heavy infection with B.diphtheriae might produce a carrier condition even where the pathological conditions present were slight. In all cases, however, the presence of pathological conditions appears to be almost an essential factor in inducing the carrier condition. The very few carriers who did not show the presence of such abnormalities in the nose, throat or ear might have had these abnormalities present at an earlier stage in convalescence. Such cases never remained carriers for lengthy periods. The importance of this finding is brought out when the treatment of the carriers comes to be considered. As the presence of

pathological abnormalities appears to be the most important factor, these should be looked for early in convalescence, at least in the first four weeks. When they are present in any case still harbouring K.L.B., they should be remedied at once, however slight they may be. If this were done the duration of the carrier state would undoubtedly be shortened very considerably.

The present work which is being carried out at the North-Eastern Hospital in typing serologically strains of the K.L.B. isolated from carriers will, it is hoped, serve to show whether or not this is a definite "carrier type" of organism. If such were the case, the difficulties met with in the carrier problem would be greatly lessened. At present, however, there is no evidence to support this. Therefore, one must still assume that the carrier condition is not in any way a function of the micro-organism.

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CONCLUSIONS.

The following are the conclusions which I arrived at after having completed the investigation:-

(1). That the most important carrier is the one with Klebs-Loeffler bacilli present in the nose, though the most difficult carrier to treat is the one with Klebs-Loeffler bacilli present in discharge from the ear.

(2). That the flora found in the noses and throats of carriers does not differ qualitatively from that found in the noses and throats of non-carriers, but that quantitively the flora is much more extensive in carriers.

(3). That this statement applies much more to the nose flora than it does to the throat flora.

(4). That pathological conditions are much more commonly found in the noses and throats, and especially in the noses, of carriers than in convalescent diphtheria patients who do not become carriers.

(5). That the presence of these pathological conditions in the noses and throats of carriers is co-existent with the extensive flora, and in most cases appears to be a necessary factor in the production of the carrier condition in diphtheria patients.

(6). That the carriers who have K.L.B. present only in the throat readily respond to treatment, and that the best form of treatment in these cases is the removal of the tonsils and adenoids.

(7). That the carriers with K.L.B. present in the nose are more difficult to treat, and that the best method of attack in these cases is the elimination of the extensive flora by means of nasal douching, autogenous vaccines or a combination of these two forms of treatment.

(8). That nasal douching is the most successful form of treatment in these cases.

(9). That the removal of the tonsils and adenoids is not successful where K.L.B. are present to any extent in the nose.

(10). That carriers who have become negative are liable to re-infection and that this liability remains for a considerable period.

(11). That carriers have a considerable amount of antitoxic substances in the blood.

(12). That the Klebs-Loeffler bacillus in carriers does not appear to be living in the actual tissues of the body but rather appears to be existing in the products of inflammation produced by co-existing organisms. It is thus carrying on an extra-corporeal existence.

(13). That pathological abnormalities should be looked for

early in convalescence after diphtheria.

(14). That any necessary treatment should be commenced within six to eight weeks of the onset of the original attack of diphtheria unless definitely contra-indicated by the presence of complications.

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In conclusion, I wish to thank Dr. F.H. Thomson, Chief Medical Officer to the Metropolitan Asylums Board's Infectious Hospitals service and Medical Superintendent of the North-Eastern Hospital, and Dr. J.E. McCartney, Director of the Board's Research and Pathological Services, for the unfailing courtesy and assistance which I received from them throughout the whole of the investigation. I should also like to thank Dr. W. Mair, Assistant Director of Research and Pathological Services, for many helpful suggestions given to me during the course of the investigation.

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APPENDICES.

APPENDIX I.

GENERAL TABLES.

These tables were drawn up from data collected during the investigation.

In some cases the desired details could not be obtained. In addition, such cases as "non-virulent carriers" and cases in which any doubt existed as to the duration of the carrier state, have been omitted from consideration.

Age incidence of the carriers (details of 314 cases).

Under 1 year ... ... (3, 3, 9, 9, 9, 9, 10 and 11 months respectively).	8 cases
1 - 5 years	199 cases
5 - 10 years	78 cases
10 - 15 years	18 cases
Over 15 years ... ... (18 years, 4; 19 years, 3; 20 years, 2; 23 years, 1; 27 years, 1.)	11 cases

Of the 11 cases over 15 years of age, 6 were nurses.

* * * * *

Sex incidence of the carriers (details of 327 cases).

Males	197 cases,
Females	130 cases.

* * * * *

The site of carrying (details of 233 cases).

No. of cases in which K.L.B. were present in the	throat only	33
" " " " " " " " " "	nose "	21
" " " " " " " " " "	nose and throat ...	143
" " " " " " " " " "	nose ...	164
" " " " " " " " " "	ear ...	36
		233

Details of ear cases.

No. of cases in which bacilli were present in the ear, nose and throat ...	24
No. of cases in which bacilli were present in the ear and nose	3
No. of cases in which bacilli were present in the ear and throat	3
No. of cases in which bacilli were present in the ear only	6
	36

* * * * *

Duration of the carrier state. (excluding all cases still in hospital).

(1). According to age.

In cases under 1 year of age	33 weeks
In cases of 1 - 5 years of age...	22.7 weeks
In cases of 5 -10 years of age	22.5 weeks.
In cases of 10 - 15 years of age	18 weeks
In cases of over 15 years of age	17.8 weeks.

(2). <u>According to site of carrying.</u>

In all cases (231 cases)	24.9 weeks
In cases with K.L.B.in the throat only(33 cases)	16.6 weeks
In cases with K.L.B.in the nose only (21 cases)	18 weeks
In cases with K.L.B.in the nose and throat (142 cases)	26.9 weeks
(The average duration of the cases in which K.L.B. were present in the nose was therefore ... ... 25.8 weeks).	
In cases with K.L.B. in the ear only (35 cases)	29.6 weeks

<u>Details of the ear cases.</u>

Average duration of 25 cases with K.L.B. in the ear, throat and nose	32.5 weeks.
Average duration of 3 cases with K.L.B. in the ear and nose	29.6 weeks.
Average duration of 3 cases with K.L.B. in the ear and throat	22.6 weeks.
Average duration of 4 cases with K.L.B. in the ear only...	16.5 weeks.

* * * * *

<u>Distribution of the carriers according to the situation of the carrier state</u> (details of 285 cases).

No. of cases in which the carrier state existed for 12 weeks	21
No. of cases in which the carrier state existed for 12 - 24 weeks	161
No. of cases in which the carrier state existed for 24 - 36 weeks	72
No. of cases in which the carrier state existed for 36 - 48 weeks	17
No. of cases in which the carrier state existed for more than 48 weeks	14

(Details as follows:- excluding two cases still in hospital -
49, 51, 52, 52, 54, 57, 60, 65, 67, 73, 86 and 91 weeks.)

N.B. One of the cases still in hospital has been a carrier for 124 weeks. This case has a cleft palate, the curative operation being unfinished.

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Types of cases on admission (details of 313 cases).

Faucial diphtheria	132 cases
Nasal diphtheria	30 cases
Nasal and faucial diphtheria ...	32 cases
Bacteriological diphtheria	20 cases
Laryngeal diphtheria	2 cases
Faucial and laryngeal diphtheria ..	2 cases
Aural diphtheria	4 cases
Nasal, faucial and laryngeal diphtheria	1 case
Clinical diphtheria	2 cases
"Rhinorrhoea, K.L.B"...	9 cases
"Otorrhoea, K.L.B"	1 case
"Diphtheria carriers"	5 cases
Scarlet fever	55 cases
Measles	11 cases
Rubella	1 case
Chicken-pox	1 case
Whooping cough	4 cases
Diphtheria and measles	1 case.

In the great majority of cases who entered hospital with diseases other than diphtheria and who later developed diphtheria, the first appearance of infection with K.L.B. was rhinorrhoea. A few developed otorrhoea. Some developed

clinical nasal or faucial diphtheria. The large number of cases who entered hospital with scarlet fever and who later developed diphtheria and became carriers (55 cases) should be noted. Likewise, the relatively large number of carriers who originally had nasal or nasal and faucial diphtheria should be noted.

* * * * *

The following table shows in tabular form the results of the treatment of carriers by means of vaccines, nasal douching and the removal of tonsils and adenoids. All cases who did not complete the full course of treatment are excluded.

RESULTS OF TREATMENT.

Form of Treatment.	No.of cases treated	No.and percentage of immediate cures.	No.and percentage of delayed cures.	Total No. and percentage of cures.	No. and percentage of failures.
Vaccines	38	10 = 26.4 per cent	6 = 15.7 per cent	16 = 42.1 per cent	22 = 57.9 per cent
Nasal douching	55	23 = 41.8 per cent	20 = 36.2 per cent	43 = 78 per cent	12 = 22.2 per cent
Vaccines & nasal douching combined	7	3 = 42.9 per cent	1 = 14.2 per cent	4 = 57.1 per cent	3 = 42.9 per cent
Removal of tonsils and adenoids.	60	33 = 55 per cent	2 = 3.3 per cent	35 = 58.3 per cent	25 = 41.7 per cent.

The "delayed cures" include all cases which took more than three weeks to clear up, and also relapses and cases in which a second course of treatment was necessary.

It must be remembered that the cases chosen for treatment were the most persistent and chronic carriers obtainable.

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APPENDIX II.

FOREIGN BODY IN THE NOSE.

A carrier who had strongly positive nose cultures was found to be harbouring a stump of lead pencil in one of his nostrils. The piece of pencil was lodged far back in the nose, but was removed without difficulty. This foreign body appeared to have been present in the nose for a very considerable period, and the child could give no account of how or when it had got there. The nose cultures were carefully examined after the removal of the foreign body, but they showed no sign of becoming negative. Later, this case cleared up after having had a vaccine.

This was the only case of its kind which was met with during the investigation.

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APPENDIX III.

VIRULENCE TESTS.

I have, in the introduction to this thesis, stressed the importance of the virulence test, and have given in some detail the method which was adopted to carry out this test. I now propose to give the results which were obtained. It was not the purpose of this investigation to carry out any special work on the virulence of the organisms carried, except in so far as this point affected the ultimate result of the work done. Many interesting facts, however, were ascertained.

I carried out the great majority of these virulence tests myself, having obtained the necessary licence to do so. Later, when the Northern Group Laboratory opened for work in May,1926, these tests were mostly carried out there. Even then, however, I still performed the tests myself in cases which called for special investigation. It was considered desirable to have the virulence established in the case of any convalescent diphtheria patient whose stay in hospital was approaching twelve weeks. Therefore, all the infectious diseases hospitals under the Metropolitan Asylums Board were instructed to have these tests carried out when necessary. In some cases, however, this was not done, and so some of the cases which were admitted

to the North-Eastern Hospital as carriers had had no virulence tests performed. In many of these cases, the carriers had no positive cultures, or only one or a few scanty positive cultures after admission to the North-Eastern Hospital, and so no organisms were obtainable upon which the virulence test could be performed. These cases, however, were mostly of little or no importance. In dealing with the cases which were treated by the special methods adopted during the investigation I have enumerated the number of cases in which the virulence was established. These are undoubtedly the most important cases. Many carriers had virulence tests repeated several times at varying intervals, where such was thought necessary.

SUMMARY OF RESULTS.

(1). 259 carriers had virulence tests carried out on the bacilli isolated, in the manner already described.

(2). 237 of these strains were found to be virulent K.L.B. (These results include the cases already dealt with in describing the organisms isolated after the removal of tonsils and adenoids).

(3). 7 of these carriers were found to be carrying non-virulent organisms and were discharged from hospital.

(4). 4 carriers were found to be carrying virulent and non-virulent organisms at the same time. One of these cases

is interesting. This was a boy, aged 14 years, who had bacilli morphologically resembling K.L.B. in the nose, throat and ear. On the 6th May, 1927, virulent bacilli were isolated from his nose and non-virulent bacilli from his throat and ear. On the 26th June, 1927, non-virulent bacilli were again isolated from his throat and ear, and on the 2nd July, 1927, non-virulent bacilli were also isolated from his nose. He was then discharged. Three months later this same boy was admitted to the Eastern Fever Hospital, reputed to be carrying K.L.B. Cultures from the nose and throat were examined at the Northern Group Laboratory by Dr. Mair, and then organisms morphologically resembling true K.L.B. were seen. These proved on isolation to be saccharose-fermenting diphtheroids.
(5). 6 carriers were found, who at different times carried virulent and non-virulent bacilli. One of these is especially interesting. This was a boy who had been found, on admission to hospital, to be carrying virulent bacilli in the nose. The virulence test was repeated, and he was then found to be carrying non-virulent bacilli in the nose. On the 7th May, 1927, bacilli were isolated from the left and right nostrils, and these were again found to be non-virulent. I then decided to investigate this case more fully myself. I succeeded in isolating 7 strains of bacilli resembling K.L.B.

from the nose. These were tested on the 24th May, 1927, and all 7 were found to be virulent. Very shortly after this the boy had his tonsils and adenoids removed, and had no further positive cultures.

(6). In 5 cases organisms morphologically resembling K.L.B. were isolated, but the organisms fermented saccharose and so were not considered to be true diphtheria bacilli.

(7). Many cases carried true K.L.B. and diphtheroids, notably Hofmann's bacillus, at the same time. I noted in several cases that when the K.L.B. disappeared from the cultures the Hofmann's bacillus remained until the case was discharged.

CONCLUSIONS.

These cases show the importance of the virulence test, and also emphasize the importance of having the test repeated at intervals. If this is not done, "non-virulent carriers" may be missed and kept in hospital much longer than is necessary. It would seem wise to have the virulence established in all cases whose cultures are still positive after four weeks in hospital.

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APPENDIX IV.

KLEBS-LOEFFLER BACILLUS IN THE SOCKET OF A DECAYED TOOTH.

One of the carriers under my care was a boy of five years of age whose nose and throat cultures were persistently positive. This boy had very decayed teeth and it was thought that these might be acting as foci for the growth of the K.L.B. I, therefore, took cultures from the region of the gum around the base of six of the most decayed teeth. I obtained yeasts from all of the teeth sockets, and, in addition, I succeeded in growing K.L.B. from the area surrounding a decayed right upper incisor. Shortly after this, the services of a dentist were obtained to remove the decayed teeth. After the teeth had been removed, however, the cultures from the nose and throat still remained persistently positive, and the child did not clear up until he was put upon nasal douches.

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GRAPHS

AND

PHOTOGRAPHS.

FIG.1.

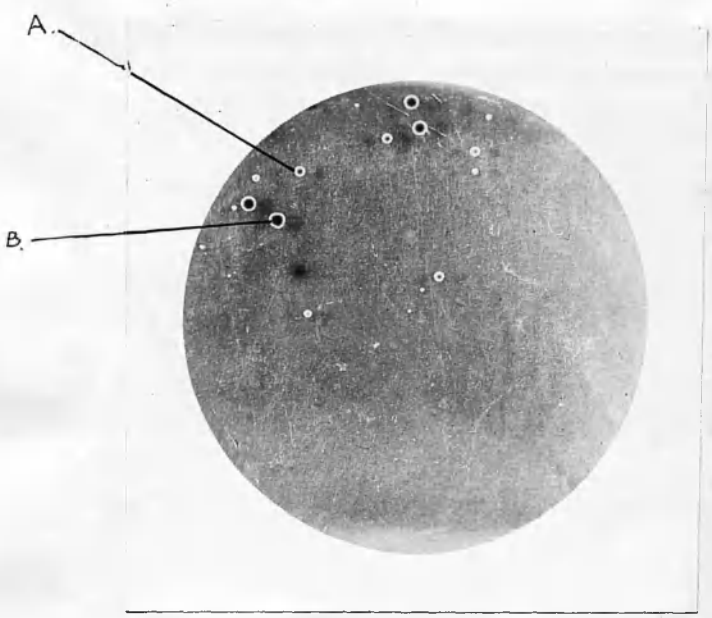


Fig.1. Showing 48 hour culture of K.L.B. on tellurite-serum-agar. Colonies of the diphtheria bacillus grown on this medium appear first white with a small grey centre. Later, the centre becomes darker, but the colony is never so black as that of a coccus. The wide white periphery as compared with the dark centre is an important point in the recognition of the colony. In addition, there is a gradual fading in colour from the dark centre to the white outer portion. Magnification - 1.25 diameters.

A. Young colony.
B. Older colony.

FIG.2.

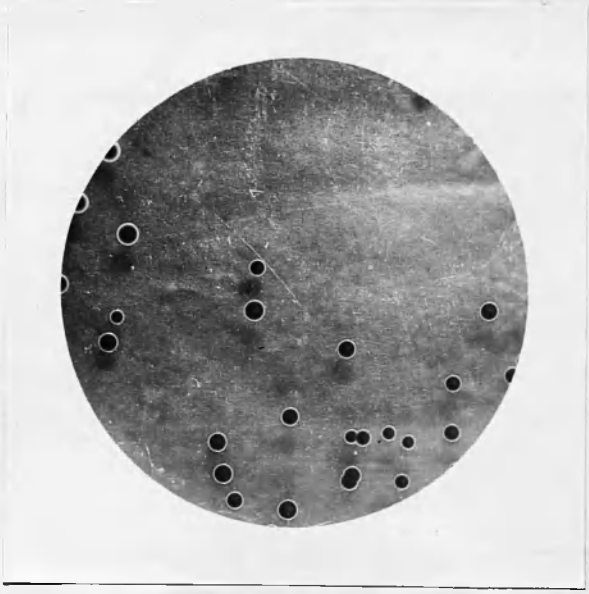


Fig.2. Showing 48 hour culture of staphylococcus on tellurite-agar.
Note that the colonies have an intense black centre, with a very narrow lighter periphery. This periphery is very sharply defined and should be compared with those seen in Fig.1. Also compare the size of the colonies with those of the diphtheria bacillus shown on Fig.1.
Magnification - 1.25 diameters.

FIG.3.

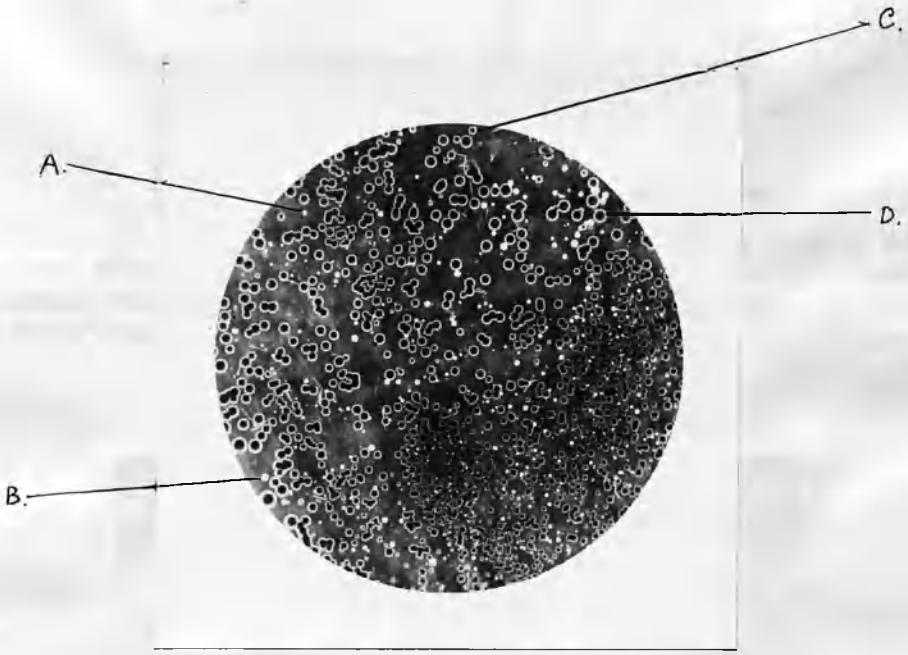


Fig.3. Showing 48 hour mixed culture of Hofmann's bacillus and staphylococcus on tellurite-agar. The Hofmann's bacillus grows in the form of a small white or greyish white colony with little or no darkening in the centre, whereas the staphylococcus shows the usual black centre and narrow white periphery.
Magnification - 1.25 diameters.

A. Young colony of Hofmann's bacillus
B. Older colony of Hofmann's bacillus.
C. Young colony of staphylococcus
D. Older colony of staphylococcus.

FIG.4.

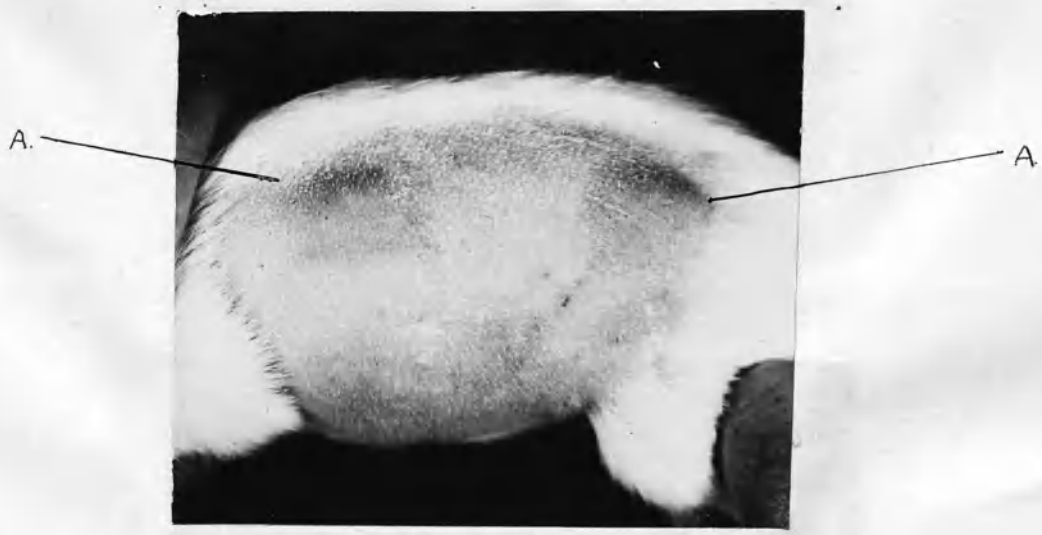


Fig.4. Flank of a guinea pig showing results 24 hours after a virulence test carried out by the intradermal method. The areas marked "A" show the typical reactions produced by the injection of virulent cultures of K.L.B.("positive reactions")

FIG.5.

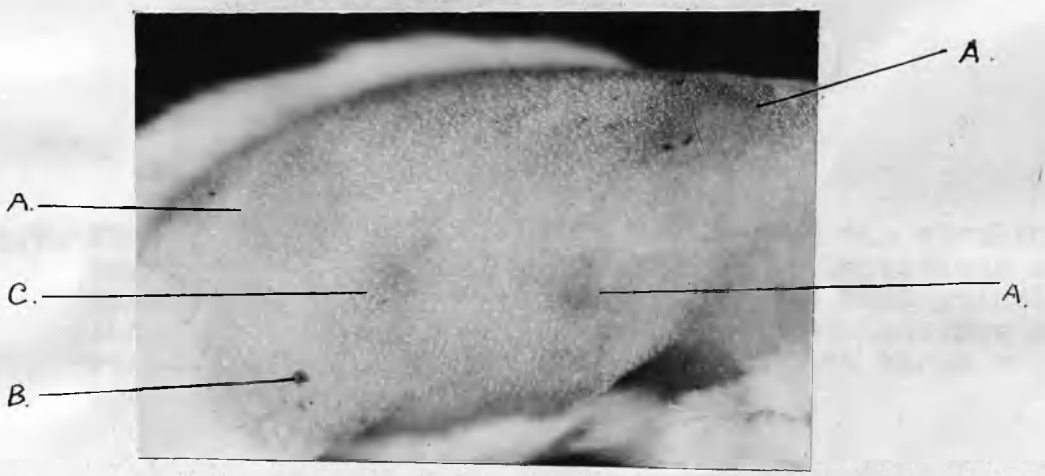


Fig.5. The opposite flank of the guinea pig shown in Fig.4. The three areas marked "A" show the reactions produced 24 hours after the intradermal injection of virulent cultures of K.L.B. The area marked "B" indicates an ink mark made at the site of injection of an avirulent culture of K.L.B., and shows the complete absence of any reaction ("negative reaction"). The area marked "C" shows a reaction the result of which was doubtful after 24 hours. The appearance of this last reaction illustrates the importance of waiting 48 hours before taking a final reading.

FIG.6.

Fig.6. Flank of the control guinea pig used in the virulence test 24 hours after inoculation. The injections were made with the same cultures used on the test guinea pig shown in Fig.5. Note the almost complete absence of reactions after 24 hours as compared with those seen in the test guinea pig.

FIG.7.

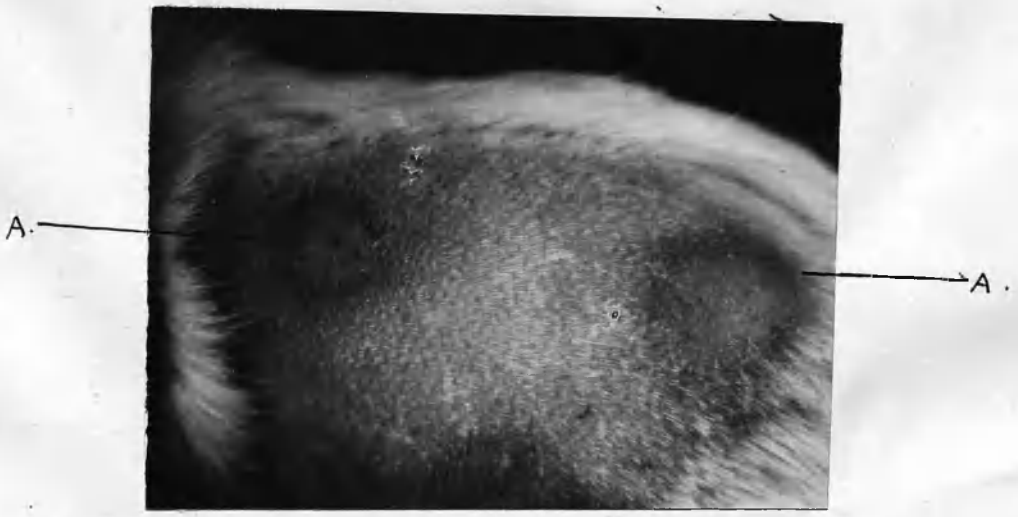


Fig.7. Reactions shown in Fig.4., 48 hours after the virulence test had been carried out. The areas marked "A" indicate two very well-marked "positive reactions", produced by virulent cultures.

FIG.8.

Fig.8. Reactions shown on Fig.5., 48 hours after the virulence test had been carried out. The two areas marked "A" show well-marked "positive reactions", produced by virulent cultures. The area marked "A1" shows a less well-marked "positive reaction". The area marked "B" indicates the site of injection of a non-virulent culture ("negative reaction"). The area marked "C" also shows a "negative reaction". Note that the result of this reaction, as shown on Fig.5. was doubtful after 24 hours.

FIG.9.

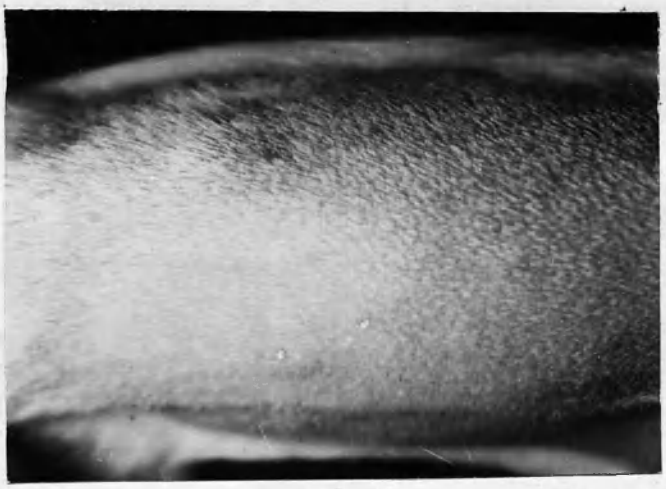


Fig.9. Flank of the control guinea pig used in the virulence test 48 hours after inoculation. Note the complete absence of reactions.

FIG.10.

Fig.10. Section of left tonsil stained by haematoxylin-eosin, showing the presence of inflammatory reaction on the surface of the tonsil. Magnification - 55 diameters.

FIG.11.

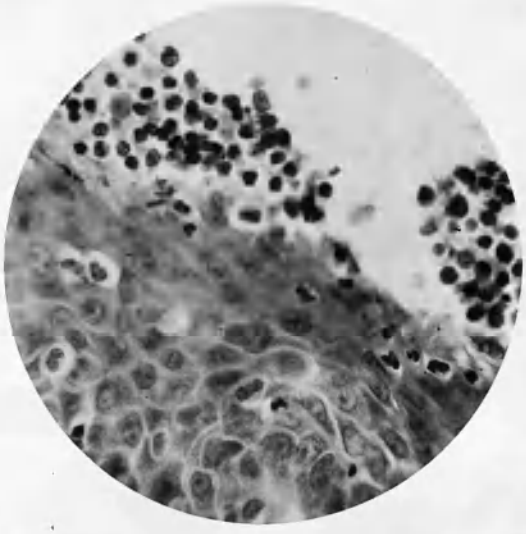


Fig.11. The same tonsil as shown in Fig.10, under a magnification of 450 diameters. Note the presence of the inflammatory exudate lying upon the surface of the epithelium.

FIG.12.

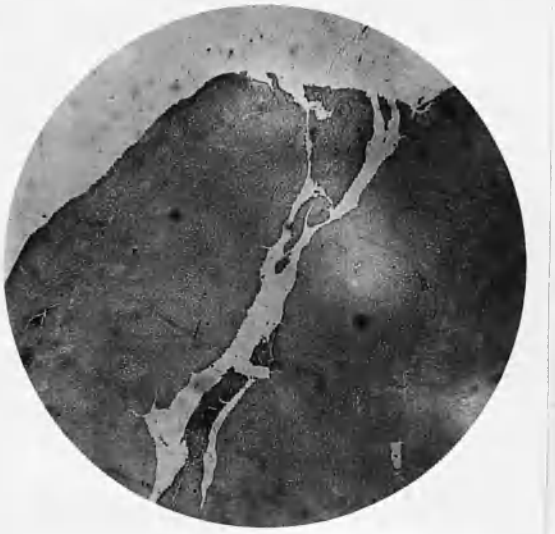


Fig.12. Section of adenoid tissue stained by Twort's method showing the presence of a deep crypt. Magnification - 12.5 diameter.

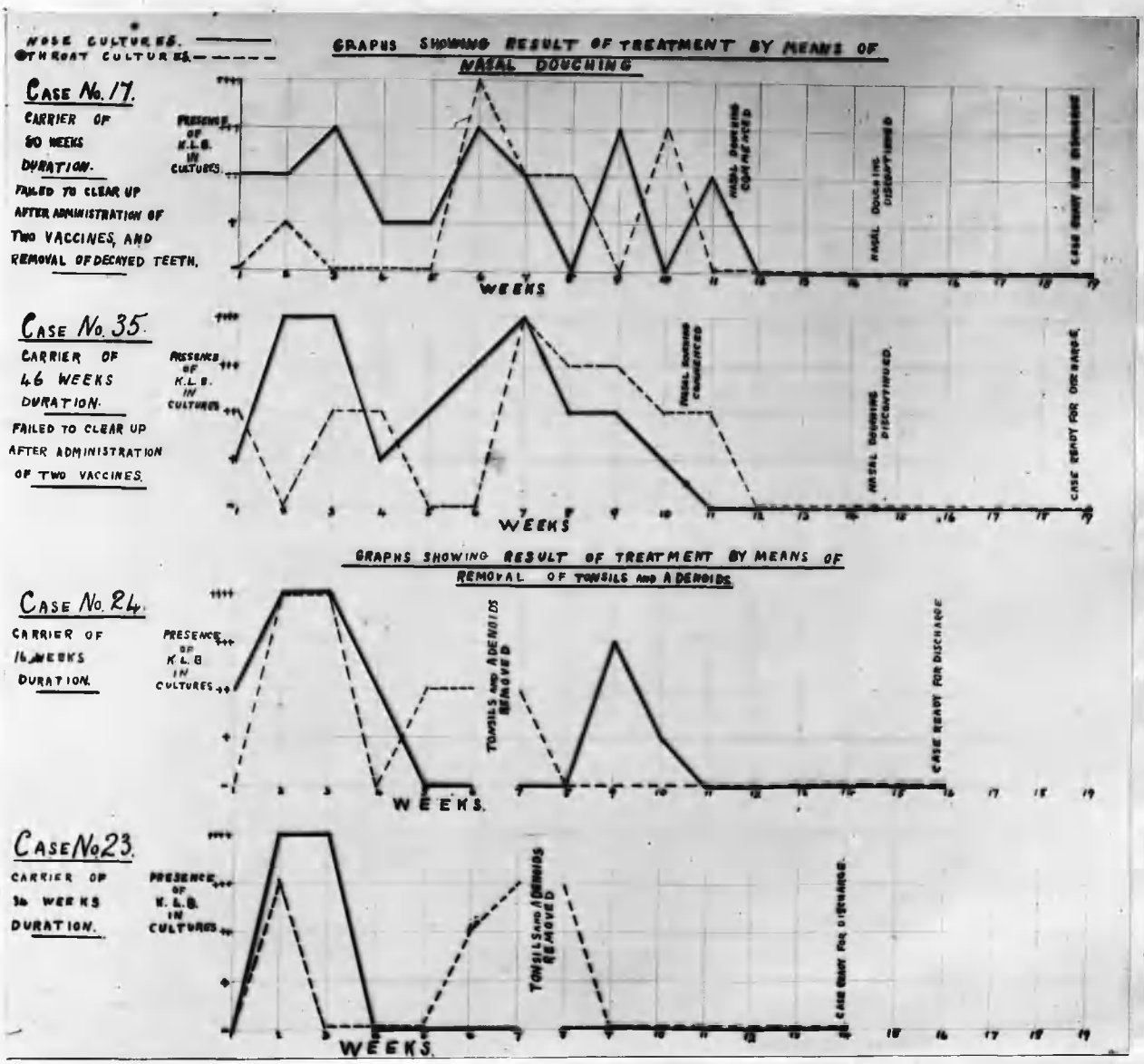
FIG.13.

Fig.13. Same section as above under a magnification of 55 diameters.

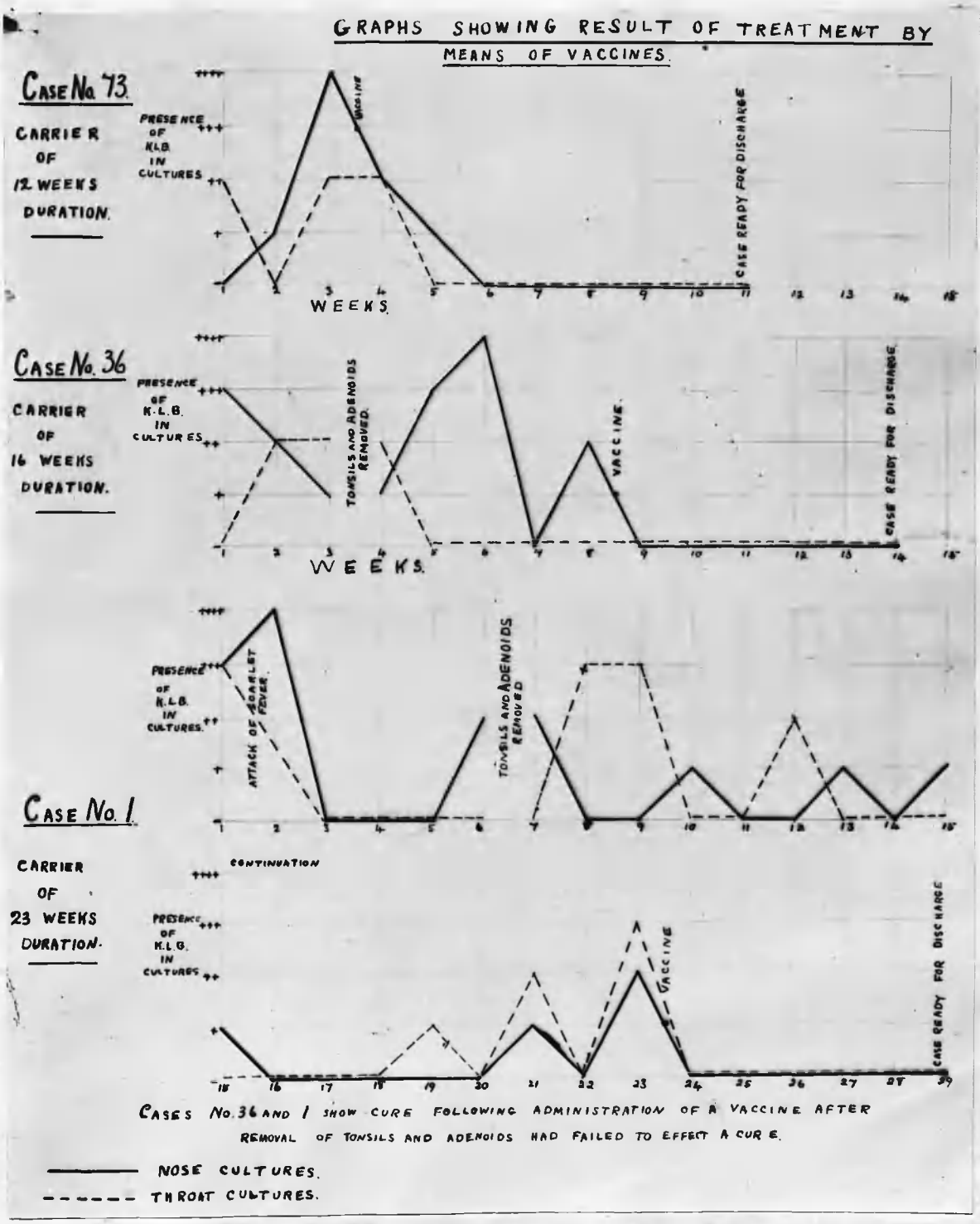
FIG.14.

Fig.14. Section of left tonsil stained by Giemsa's method, showing the presence of crypts.
Magnification - 12.5 diameters.

Graphs 1 - 4 showing results of treatment (1) by means of nasal douching, and (2) by means of removal of tonsils and adenoids.

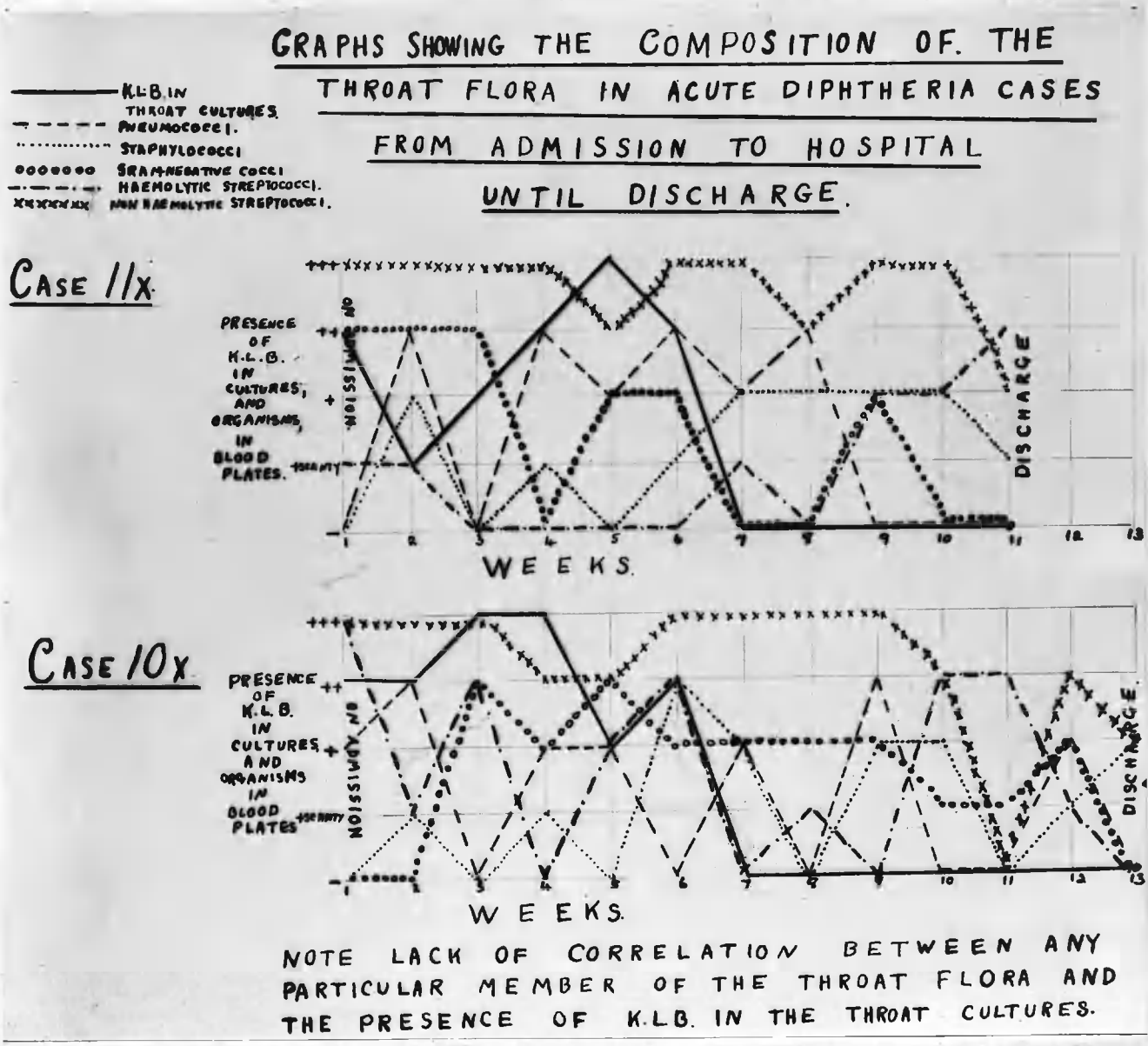


Graphs 5 - 7 showing result of treatment by means of vaccines



CASES No. 36 AND 1 SHOW CURE FOLLOWING ADMINISTRATION OF A VACCINE AFTER REMOVAL OF TONSILS AND ADENOIDS HAD FAILED TO EFFECT A CURE.

Graphs 8 & 9 showing the composition of the throat flora in acute diphtheria cases from admission to hospital until discharge.



Graphs 10 - 13 showing the composition of the nose flora in acute diphtheria cases from admission to hospital until discharge.