ON THE BACTERIAL FLORA OF DIPHTHERIA CARRIERS, DIPHTHERIA PATIENTS, AND NORMAL SCHOOL-CHILDREN.

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(With 6 Graphs.)

During a recent investigation on the carrier problem as it occurs in diphtheria, which was carried out (under the auspices of the Scientific Advisory Committee of the Metropolitan Asylums Board) at the Northern Group Laboratory, North-Eastern Hospital, one of the first subjects considered was that of the bacterial flora of the carriers under examination, and of an adequate series of controls. It was suggested that possibly the flora of the carriers might differ (apart altogether from the presence of the Klebs-Loeffler bacillus) from that found in non-carrier persons *of the same age*. It was thought that some pathogenic organism might be found to be constantly present in the nose or throat flora of carriers and not in controls, and that this might be exerting an influence on the Klebs-Loeffler bacillus by allowing it to survive in the infected region for prolonged periods. If such were the case it was hoped that an elimination of the organism or organisms concerned would go a long way towards clearing up the carrier condition.

A series of very illuminating papers on this subject has been recently published in America 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 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 dealing with the dissemination of bacteria in the upper respiratory air passages, Bloomfield 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

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rapid transport of the bacteria towards the oesophagus. 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 in two further papers, and finds 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, 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 as follows: "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."

Bloomfield's papers are quoted somewhat fully as they have a direct bearing upon the subject under discussion, while the standard of work done was such as to render the conclusions arrived at very valuable.

Bearing these points in mind a series of carriers and controls was collected and the flora of the nose and throat examined.

THE THROAT FLORA.

The first site to be investigated was the throat. Thirteen strongly positive carriers were selected for investigation. Material, obtained from the throats by swabbing, was inoculated on blood-agar plates. These were made by preparing a saline suspension of the material obtained by swabbing and mixing various quantities of that suspension into the fluid blood-agar, which was then poured into a Petri dish. 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 from hospital. Nose and throat cultures were taken simultaneously and examined for the presence of diphtheria bacilli. Fifteen diphtheria patients were dealt with in this way, and altogether 220 blood plates were made and examined. There were 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 once a week, and swab the throats of 55 apparently healthy school-boys. Blood plates were made from the swabs, and so a group of 110 plates was obtained for comparison.

The majority of the carriers examined were children of between five and fifteen years of age. The diphtheria patients and the school-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 as follows:

There did not appear to be any direct relationship between the flora of the throats 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 non-haemolytic), *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.

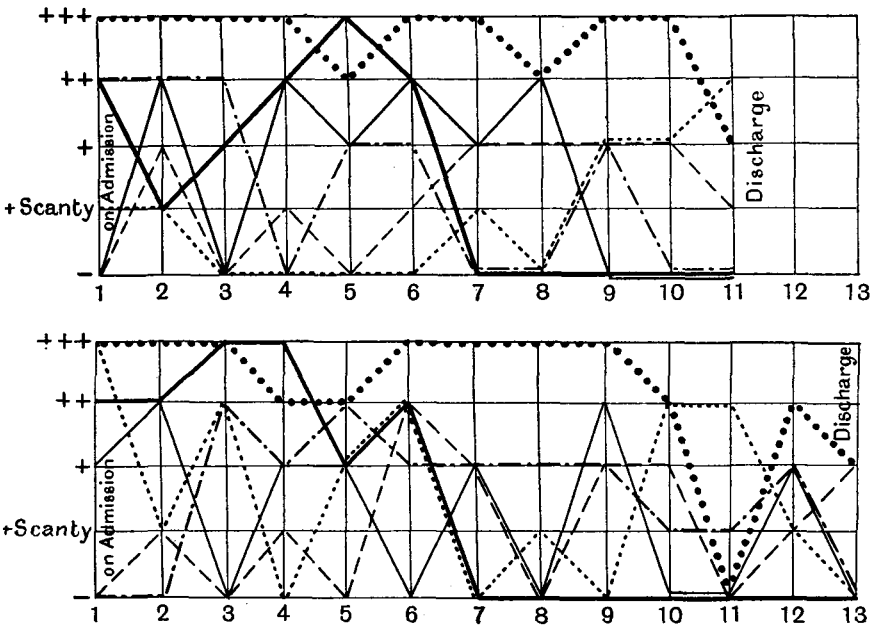
As regards the carriers, there did not appear to be any organism invariably present or absent in the flora of the throats examined, nor did the flora change greatly if the case showed signs of clearing up. Similarly, the flora of acute diphtheria cases remained relatively unchanged throughout convalescence, irrespective of the severity of the original disease or of the length of convalescence. Table I shows the type of organisms found in each group.

It could not be stated, therefore, that any particular organism found in

Table I. *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) group of 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	Vary 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	Quite commonly present. Diminishing as convalescence proceeds

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.



Graphs I and II. Showing the composition of the throat flora in two acute diphtheria cases from admission to hospital until discharge. The divisions of the ordinates represent the presence of Klebs-Loeffler bacilli in cultures and organisms in blood plates, while the divisions of the abscissae represent weeks. The lack of correlation between any particular member of the throat flora and the presence of the Klebs-Loeffler bacillus in the throat cultures should be noted. See Key to different lines under Graph VI, p. 235.

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the throat was exercising a beneficial or an antagonistic influence on the Klebs-Loeffler bacillus.

Graphs I and II show the apparent lack of relationship between the results of the cultures as regards the presence of the Klebs-Loeffler bacillus and the type of organisms present in the throat.

The Nose Flora.

The flora of the nose next received attention, the same procedure as used in the examination of the throat flora being employed. 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 proliferant. Very few clean or even 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

Table II. *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) group of 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 bacillus	Commonly present	Occasionally present	Never present	Occasionally present. Diminishes during convalescence

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Beta haemolytic streptococcus in their plates. This appeared, however, to be merely 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.

Tables II and III 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 III.

(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.

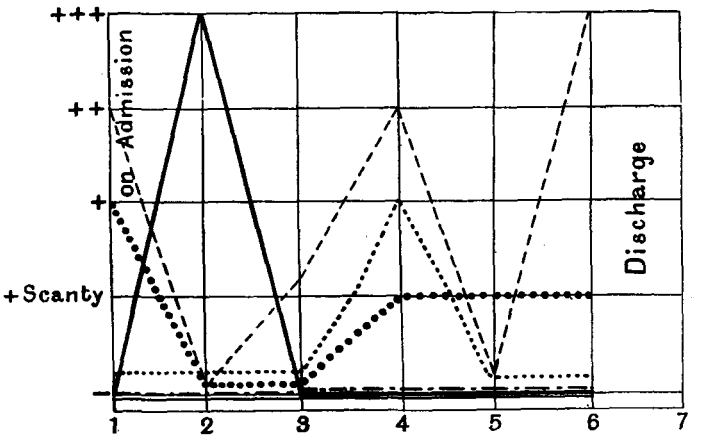
(3) *Results obtained from the examination of blood plates made from the noses of 10 boys attending one class at Duncombe Road Schools.*
Results. 6 plates sterile.
1 plate practically sterile.
Staphylococcus albus present in 3 cases.
Non-haemolytic streptococcus present in 1 case.

(4) Case 10 X. (*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.

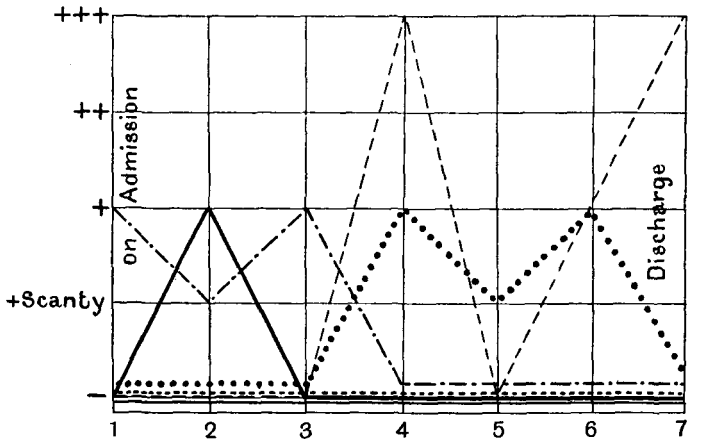
(5) Case 11 X. (*Clinical diphtheria, nose and throat positive.*) Blood plates made from the nose on 6 occasions.
Results. Beta haemolytic streptococcus present on 1 occasion.
Non-haemolytic streptococcus present on 2 occasions.
"Viridans" present on 1 occasion.
Staphylococcus albus present on 4 occasions.
Staphylococcus aureus present on 1 occasion.
Blood plates sterile on 1 occasion.

(6) Case 15 X. (*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.

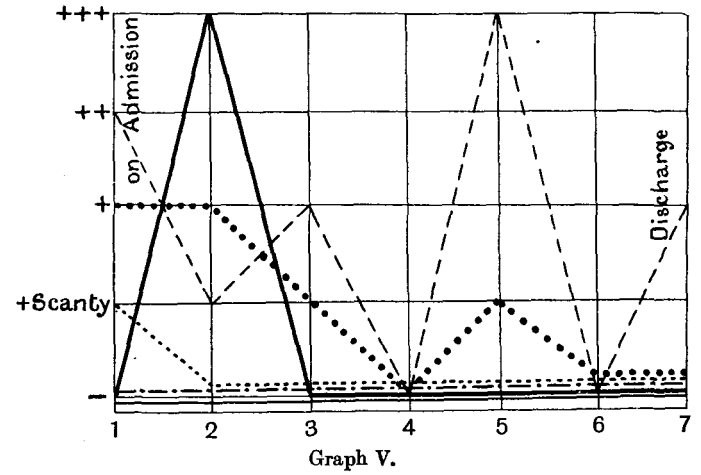
The cases enumerated in Table III represent the type of organism isolated in each group dealt with. Exceptions were found, but, on the whole, these cases are fully representative of the group to which they belong.



Graph III.



Graph IV.



Graph V.

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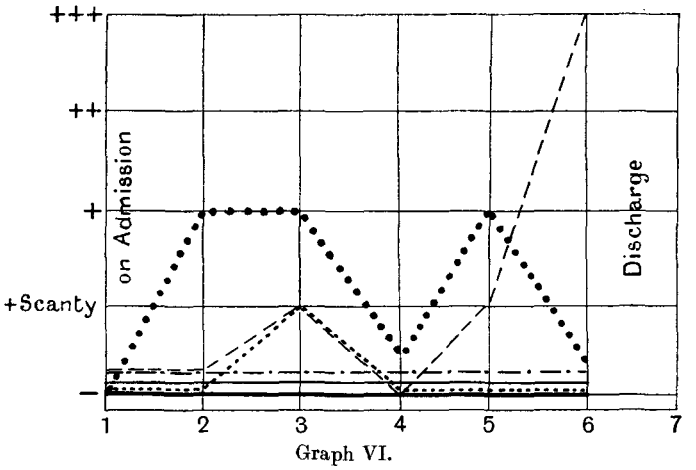

Graph VI.

Graphs III–VI. Showing the composition of the nose flora in four acute diphtheria cases from admission to hospital until discharge. The divisions of the ordinates and abscissae are as in Graph I and the same lack of correlation between any particular member of the nose flora and the presence of Klebs-Loeffler bacilli in the nose cultures should be noted.

a Klebs-Loeffler bacilli in nose cultures.
b Pneumococci.
c Staphylococci.
d Gram-negative cocci.
e *Haemolytic streptococci.*
f Non-haemolytic streptococci.

Blood plates were also inoculated with material obtained from chronic carriers and incubated under *anaerobic conditions*. These were controlled 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.

1. The throat flora of carriers does not differ greatly either quantitatively or as regards the individual component members from that of non-carrier persons of the same age.

2. The nose flora of carriers is made up of the same organisms as compose the flora of non-carriers, but these organisms are present in much greater numbers in the noses of carriers.

This last 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 seemed wise 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 noses with an alkaline douche.

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

It is outside the scope of this paper to deal with the results obtained from these forms of treatment—they have been embodied in a recent paper of mine (1928). It will suffice to say here that they were quite successful and that the disappearance from the noses of the proliferant flora was accompanied by a marked decrease in the duration of the carrier condition.

Furthermore, an attempt was made to correlate the presence of abnormalities or pathological conditions in the noses of carriers, with the increased flora, by having these noses examined by a competent nose and throat surgeon. As might be expected, it was found that the presence of such abnormalities or pathological conditions was always accompanied by an increase in the local flora and so the methods of treatment adopted were such as aimed at correcting these former conditions, by vaccines or nasal douches as already indicated. When such had been done, it was found that in almost every case the flora of the nose diminished.

For further details regarding these last observations see McCartney and Harvey (1928 *a*).

REFERENCES.

BLOOMFIELD (1921). *Johns Hopkins Hosp. Bull.* **32**, 360.
—— (1922). *Ibid.* **33**, 145.
—— (1921 *a*). *Amer. Rev. of Tuberculosis*, **4**, 11.
—— (1922 *a*). *Ibid.* **5**, 11.
—— (1922 *b*). *Amer. J. Med. Sci.* **164**, 854.
MAIR (1917). *Biochem. J.* **11**, 1.
—— (1917 *a*). *J. Path. and Bact.* **21**, 305.
HARVEY (1928). *Lancet*, ii, 58.
MCCARTNEY and HARVEY (1928 *a*). *Proc. Roy. Soc. Med. Lond.* p. 845.

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