The incidence of Diplococcus pneumonia Weichselbaum in a group of individuals at Montana State College

by Elizabeth M Carter

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the degree of Master of Science in Bacteriology at Montana State College

Montana State University

Abstract:

During the school years of 1958 to 1940,, a Survey to determine the carrier incidence of Diplocoacus pneumoniae in the throats of a group of individuals at Montana State College was carried out. Various methods of obtaining cultures were tried.

Using the results of this work and the suggestions received from other investigators as a guide, a more comprehensive survey was carried out from October 1940 through April 1941 on a group of 54 individuals.

The group was composed of some members of the college faculty, some members Of the clerical staff, but mostly it was made up of college students. Two methods, the gargle and the swab methods with modifications developed by the author, of obtaining cultures of Dlplocpccus pneumoniae were used in order that the relative merits of each might be determined.

Of the 54 individuals tested, 75.9 per cent (41) were carriers of Diplococcus pneumoniae at one time or another during the survey.

Experimental evidence is presented which indicates that neither the gargle nor the swab method can be depended on to secure cultures from 100 per cent of the carriers, and that both methods should be used simultaneously when determining the carrier incidence. Evidence is also presented which shows that beef heart infusion broth enriched with beef serum gives a much higher per cent of positive cultures than does the unenriched broth.

The individuals tested in this survey were grouped using the classification of the author.

Nineteen specific types of DiplococCus pneumoniae were found during this survey. Type 5 was the most common; types 1 and 2 were never found.

"...It is easy to make mistakes in this field of investigation; easier, perhaps, than to acknowledge them. And believing, as I do, in human fallibility, I have no hesitation in questioning the conclusions of the most illustrious workers in the field of microbiology, if they are in conflict with my own observations. On the other hand, if, upon fuller investigation, I am convinced that I have been mistaken in regard to this or any other question, I shall feel no hesitation in following the example of Pasteur in making a public announcement of my error."

--Sternberg (1885)

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ABSTRACT

During the school years of 1938 to 1940, a survey to determine the carrier incidence of Diplococcus pneumoniae in the throats of a group of individuals at Montana State College was carried out. Various methods of obtaining cultures were tried.

Using the results of this work and the suggestions received from other investigators as a guide, a more comprehensive survey was carried out from October 1940 through April 1941 on a group of 54 individuals. The group was composed of some members of the college faculty, some members of the clerical staff, but mostly it was made up of college students. Two methods, the gargle and the swab methods with modifications developed by the author, of obtaining cultures of Diplococcus pneumoniae were used in order that the relative merits of each might be determined.

Of the 54 individuals tested, 75.9 per cent (41) were carriers of Diplococcus pneumoniae at one time or another during the survey.

Experimental evidence is presented which indicates that neither the gargle nor the swab method can be depended on to secure cultures from 100 per cent of the carriers, and that both methods should be used simultaneously when determining the carrier incidence. Evidence is also presented which shows that beef heart infusion broth enriched with beef serum gives a much higher per cent of positive cultures than does the unenriched broth.

The individuals tested in this survey were grouped using the classification of the author.

Nineteen specific types of Diplococcus pneumoniae were found during this survey. Type 3 was the most common; types 1 and 2 were never found.

INTRODUCTION

This paper presents the results of a survey by the author of a group of individuals selected on the Montana State College campus to determine the incidence of the various types of Diplococcus pneumoniae Weickselbaum in the throats of this group. The results should throw some light on the incidence of Diplococcus pneumoniae among the individuals at that institution.

It is also the object of this paper to compare two different methods of obtaining cultures from the throats of individuals and to show the relative merits of the two methods.

HISTORICAL

Incidence of the carrier rate.--Although Klebs (1875) is given credit for first seeing pneumococci, it was Sternberg (1881) who first inoculated rabbits with normal saliva and isolated the characteristic lancet-shaped diplococci which we now recognize as Diplococcus pneumoniae. Sternberg, however, did not appreciate the significance of these organisms, and it remained for Friedlander (1884) to designate these diplococci as the causative agents of pneumonia.

Since the work of Dochez and Avery (1915), it has been known that pneumococci are present in the throats of a large number of healthy individuals. These authors found that, while pneumococci of types I and II caused over 60 per cent of the cases of lobar pneumonia in adults, the incidence in the normal mouth was less than 1 per cent. They found type III and group IV to be responsible for 30 per cent of the pneumococcus

cases and to be found frequently in the mouth.

Powell, Atwater, and Felton (1926) made several surveys during the period from September 1923 to March 1924 in Boston and found the incidence of pneumococci to be as follows:

(a) Of 104 laboratory workers 70 (67 per cent) were carriers of pneumonia organisms. Type I occurred in 2 (1.9 per cent) of those examined during the survey; type II in 10 (9.6 per cent); type III in 14 (13.4 per cent); and type IV (now referred to as group IV) in 57 (54.8 per cent).

(b) Of 112 high school boys 74 (66 per cent) were carriers. Type I occurred in 7 (6.2 per cent) of those examined during the survey; type II in 6 (5.3 per cent); type III in 5 (4.4 per cent); and type IV in 66 (58.9 per cent).

(c) Of 112 medical school students 74 (66 per cent) were carriers. Type I occurred in 4 (3.5 per cent) of those examined during the survey; type II in 3 (2.6 per cent); type III in 5 (4.4 per cent); and type IV in 67 (59.8 per cent).

(d) Of 90 student nurses 48 (53 per cent) of those examined during the survey were carriers. Type I occurred in no cases; type II in 1 (1.1 per cent); type III in 8 (8.8 per cent); and type IV in 44 (48 per cent).

(e) Of the total group of 93 persons on whom 418 examinations were made, type 1 occurred in 3.1 per cent of the examinations; type II in 4.8 per cent; type III in 7.7 per cent; and type IV in 56 per cent.

Meyer (1920) found no strains of type I or II among 100 healthy individuals; only 3 strains (3 per cent) of type III; and 17 strains

(17 per cent) of group IV organisms.

Webster and Hughes (1931) reported that in an extended investigation embracing monthly examinations over a period of from two months to two and one-half years, of 105 children and adults living in New York City, pneumococci were obtained at one time or another from the nasal passages or the throat of 80 per cent of the persons studied. They also noted that the incidence of pneumococci in all of the individuals included in the study underwent seasonal fluctuations corresponding to the changes in the prevalence of coryza and sore throats in the same person. This latter observation was also reported by Longcope and Fox (1905).

Gundel (1933) reported that repeated tests had been made every four weeks for one year on the upper respiratory tract of over 100 normal subjects. Type I occurred in 0.8 per cent of those tested; type II in 0.4 per cent; type III in 6.7 per cent; and group IV in 60.0 per cent.

Schleifstein (1938) made a study of 100 normal individuals in Albany, New York, who had not been in contact with pneumonia patients and found 73.0 per cent to be carriers of pneumococci.

Bliss, McClaskey, and Long (1934) made a study of 20 subjects in the John Hopkins University Medical School, on whom 1,016 cultures to be tested for Diplococcus pneumoniae were taken over a period of two years. Of these cultures 54.5 per cent were positive for pneumococci. All but one of the subjects yielded positive cultures of Diplococcus pneumoniae at least once during the course of the investigation.

Blacklock and Guthrie (1933) found pneumococci in the throats of 58.6 per cent of the healthy children examined.

Rosenau, Felton, and Atwater (1926) reported on the work of Sailer, Hall, Wilson, and McCoy (1917) who found, in 700 examinations, 16 per cent to be carriers of pneumococci.

Incidence of multiple-type carriers.--In the surveys which have been carried out to determine the ability of healthy individuals to carry Diplococcus pneumoniae in their throats, some investigators have found more than one type to be present at the same time.

Gundel and Okura (1933) investigated the occurrence of pneumococci of more than one serological type in the same subject. Of the individuals studied, 38 per cent carried organisms of two or more types. The appearance of the new types was attributed by the authors to infection from without or possibly to the development of a type which had been suppressed by the dominance of the first type found.

MacKenzie, Tepperman, and McKee (1940) found in a rural community that 41.3 per cent of the 250 people upon whom seven surveys were made during 14 months could be classed as multiple-type carriers.

Importance of contacts.--Dochez and Avery (1915) recognized that the existence of the carrier state among healthy persons and among those recently recovered from pneumonia established a basis for understanding the mechanism by which lobar pneumonia spreads and maintains its high incidence from year to year.

In a group of 270 contacts with 28 cases of pneumonia, Rosenau, Felton, and Atwater (1926) found a consistent excess of carriers over those found in the control group. The excess appeared only in those types which were present among the 28 cases, namely types I and III.

They found that contacts of type I cases were three times as likely to carry type I organisms as are controls (8.1 per cent against 2.3 per cent). Contacts with type III cases were twice as likely to carry type III organisms as controls (17.8 per cent against 9.9 per cent). It was also pointed out by these authors that type III organisms occurred in control populations more frequently than types I and II taken together, yet in most years type III causes not more than one-sixth as much pneumonia in the United States as types I and II. Type III apparently spreads from both carriers and cases more rapidly than does type I.

Rosenau, Felton, and Atwater (1926) showed that among 220 persons exposed to 28 carriers, 9 (4.1 per cent) carriers of the homologous organism were detected. They felt that the evidence presented corroborated the assumption that carriers, as well as cases, spread pneumococci, though, as they pointed out, the cases were more prolific sources than were the carriers.

MacKenzie, Tepperman, and McKee (1940) in a study of carriers in a village of 250 people in which 5 cases of type I pneumonia had occurred within 8 weeks, found that the carrier rate of all types of Diplococcus pneumoniae was 73.4 per cent. The carrier rate of type I was 24.3 per cent. Five months after the occurrence of the last case of pneumonia, the carrier rate dropped to 5.9 per cent.

Identification of types of Diplococcus pneumoniae.--Cooper and her associates (1929 and 1932) succeeded in showing by agglutination tests that Diplococcus pneumoniae in Group IV could be subdivided into 29 different types. Previous to this work, the identification of Diplococcus

pneumoniae to types was made as follows: All organisms which failed to agglutinate with antisera for types I, II, or III and which were able to ferment inulin were placed in group IV. Cooper's work greatly aided the identification of Diplococcus pneumoniae. But when Neufeld and Etinger-Tulczynska (1933) brought forth their method for type determination, it was possible for investigators to place their organisms into the correct types to the number of 32.

Occurrence of "suspicious" cultures.--Although antisera for types 1 to 33 are now available for typing Diplococcus pneumoniae, some investigators have been unable to classify as to type all of their cultures which by other tests seem to be Diplococcus pneumoniae.

Schleifstein (1938) found that of 132 strains of pneumococci only 84 could be classified. In 15 instances, a "Quellungs" reaction was obtained in pooled sera, but the pneumococci were present in such small numbers that identification was not attempted. Thirty-three cultures were bile soluble but could not be classified as to type.

MacKenzie, Tepperman, and McKee (1940) found that 18 per cent of the carriers harbored pneumococci which did not react with any of the specific sera of types 1 to 32.

Possibility of change from one type to another.--There have been, from time to time, suggestions from various sources that one type of Diplococcus pneumoniae might change to another type in vivo. However, there seems to be some divergence of opinion.

Megrail and Ecker (1924) stated that pneumococci had a type stability when placed under conditions which caused the typhoid and other organisms

to show variability in agglutination.

Barnes and Wright (1936) mentioned that their search of the literature had failed to produce definite evidence that a virulent pneumococcus could undergo spontaneous conversion from one type to another. They did, however, say that some of their work did not prove, but did strongly suggest, that under certain routine conditions a type V pneumococcus would spontaneously change into a type II organism. They were unable to give any factor or factors which might be responsible for this instability.

Dawson (1930) made the statement that it had not been conclusively demonstrated that transformation of types among Diplococcus pneumoniae actually occurred under natural conditions.

Gundel and Okura (1933) believed that the appearance of new types in the throats of individuals studied could be attributed to infection from without or possibly to the development of a type which had been suppressed by the dominance of the first type found.

Kinds of carriers.--It has been found that those individuals who carry pneumococci in their throats are not always consistent either in the length of time they carry the organism or in the type of organism they carry.

Stillman (1916) found that persons recovering from pneumonia remained carriers for 7 to 90 days. During that time they could act as "contact carriers".

Webster and Hughes (1931) found that some people were pneumococcus-free, some were "transient" carriers (positive on single and scattered

occasions between pneumococcus-free periods), some "periodic" carriers (those from whom pneumococci of one serological type were obtained for periods of 1 to 12 weeks between pneumococcus-free intervals), and some were chronic carriers (those from whom pneumococci of one serological type were obtained for periods of three months to three years or more). Evidence was presented showing that these differences were due to a variation in host-resistance to the pneumococcus organisms.

Bliss, McClaskey, and Long (1934) attempted a classification of carriers based on repeated bacteriological examinations extending over a period of a year or more, which was a slight modification of that drawn up by Webster and Hughes (1931). After a year's study of young adults the authors divided the subjects into non-carriers and chronic carriers, the latter group including those persons who intermittently exhibited pneumococci in their throat. While the so-called intermittent carriers might or might not yield positive cultures on repeated examination, the cultures when positive were consistently of the same type of pneumococcus in any given case, indicating to these authors a chronic condition with constant bacteriological findings only as to type. Furthermore, these authors considered that their demonstration added evidence in favor of the stability of pneumococcal types in the human body. It seemed more logical to these authors to designate healthy individuals who harbor pneumococci in the nose and throat for short periods of time as temporary carriers, and those in whom organisms persisted for longer periods of time as chronic carriers. It was suggested by these authors that a further subdivision of chronic carriers into "continuous" and

"intermittent" carriers would define more accurately the condition and the possible menace of the chronic case.

MATERIALS

For growing pneumococci it was necessary for the medium used to support growth but not to cause rapid autolysis. Beef heart infusion broth which contained 0.2 per cent Na_2HPO_4 as a buffer and 2 per cent peptone was found to be very satisfactory. This broth was prepared as follows: The fat was removed from beef hearts and the meat ground in an ordinary meat grinder. To each 500 g. of meat, 1000 cc. of distilled water was added. This mixture was infused in the icebox for 12 to 18 hours. After the fat had been skimmed from the top, the infusion was boiled for ½ hour and the volume was restored with distilled water. With the aid of a meat press, the liquid was squeezed from the meat. This liquid was run through filter paper, and the volume again restored with distilled water. To each 1000 cc. of this infusion was added 20 g. of Difco peptone, 5 g. NaCl, and 2 g. Na_2HPO_4 and this mixture was heated to dissolve the peptone. After being titrated and adjusted to pH 7.6 to 7.8, the broth was boiled for ½ hour. It was then allowed to cool to 30° C. before it was filtered, tubed, and sterilized in the autoclave at 15 lbs. pressure for 15 minutes. The final pH was approximately 7.4.

To insure the best growth of the pneumococci, the beef heart infusion broth was enriched by the addition of approximately 1 per cent fresh beef serum which had previously been sterilized by the use of a Berkefeld filter and which had 1 per cent of formalin added to it.

Sodium desoxycholate solution was used in the test for solubility of the cultures (Kolmer and Boerner, 1938). It was prepared as follows: To every 100 cc. of distilled water, 10 g. of chemically pure sodium desoxycholate was added. The mixture was shaken and stirred until completely dissolved and then autoclaved for 15 minutes.

METHODS

Methods of obtaining cultures for the 1939 to 1940 survey.--In the survey carried out during the school year 1939 to 1940, several methods were tried with varying degrees of success. The first method was as follows:

Using sterile swabs, cultures were taken from the tonsilar crypts, care being taken to avoid touching other parts of the mouth. The swab was placed in beef heart infusion broth enriched with approximately 1 per cent beef serum and incubated at 32° C. for 12 to 24 hours. This culture was then streaked on blood agar plates. Cultures were taken from the nasopharynx region and treated in the same way as those from the tonsilar region. After a 24 to a 48 hour incubation period at 32° C., colonies which resembled those of Diplococcus pneumoniae were transferred to a 1 per cent dextrose broth. The dextrose broth was incubated at 32° C. for 12 hours and the Neufeld "Quellungs" reaction carried out. This method proved to be unsatisfactory since it yielded no pneumococci.

The second method, which proved to be more satisfactory than the first but not as satisfactory as the third, was as follows:

A swab was taken from the nasopharynx region and placed in beef heart infusion broth enriched with approximately 1 per cent beef serum and

incubated at 37° C. for 4 to 6 hours. One cc. of this culture was injected into the peritoneum of a white mouse. When the mouse became very sick or was just on the verge of death, he was autopsied. A culture of blood was taken from the heart and placed in a tube of enriched beef heart infusion broth. This culture was incubated at 37° C. until growth became visible. At that time, the Neufeld "Quellungs" reaction was carried out.

The third, and most successful method was as follows:

The student to be examined for the presence of pneumococci was asked to rinse his mouth, especially around the teeth, with 20 cc. of physiological saline solution (0.85 per cent). This washing was discarded. Then 20 cc. of the saline solution was used as a gargle for from 1 to 3 minutes and then expelled into a sterile container. This washing was centrifuged at 2000 r. p. m. for one hour. All the sedimented material was then suspended in 1 cc. of saline solution and injected into the peritoneal cavity of a white mouse. Six hours after injection, a sterile capillary pipette was inserted into the peritoneal cavity of the mouse and some of the fluid was withdrawn. One drop of this fluid was placed in beef heart infusion broth; another drop was smeared on a blood agar plate; and a third drop was smeared on a slide for staining.

After 48 hours incubation at 37° C., suspicious colonies were transferred to sterile beef heart infusion broth. When growth appeared in the tubes, 1 cc. portions were placed in each of three small tubes. To the first tube was added four drops of a 10 per cent aqueous solution of sodium desoxycholate; to the second tube was added ½ cc. of a 10 per cent aqueous solution of Bacto ox-gall; and the third tube was used as a control.

Smears (for the Neufeld "Quellungs" reaction) were made on glass slides of all cultures which were soluble with either or both of the above tests. Blood agar plates were streaked from all positive cultures in order to obtain a pure culture of the pneumococci.

Those mice which did not die were observed for 15 days, after which they were etherized, autopsied, and cultures taken from the peritoneal cavity and from the heart. The same procedure as described above was carried out with any cultures showing growth.

Selection of individuals for the 1940 to 1941 survey.--All but 14 of the individuals were chosen at random from those who were in Lewis Hall when the work on this survey began in October 1940. The group of 14 were girls who lived in a sorority house on the campus at Montana State College. (Individuals 1 to 9, 11 and 12, 14 to 16 in Table I)

Methods of obtaining cultures in the 1940 to 1941 survey.--In order that the two methods, swabbing and gargling, might be compared, each individual tested had his throat swabbed first and then was asked to gargle. The two procedures are described below:

The Swab Method.--With a sterile cotton swab a culture was taken from the nasopharyngial region of each individual. The swab was placed in a tube of enriched beef infusion broth and incubated at 37° C. for 6 hours. At the end of that time the broth was centrifuged at high speed (approximately 2000 r.p.m.) for 45 minutes. The supernatant fluid was poured off and the sediment suspended in 1 cc. of sterile physiological saline solution (0.85 per cent). One cc. of this suspension was injected intraperitoneally into a white mouse. Six hours later, by means of a

small capillary pipette, some fluid was withdrawn from the peritoneum. A drop of this liquid was placed in enriched beef heart infusion broth and incubated at 37° C. until sufficient cloudiness was present to run a solubility test with sodium desoxycholate. This test consisted in taking two 1 cc. samples of culture, adding 4 drops of sodium desoxycholate solution to one, and using the other as a control tube. The test was observed over a period of one hour and those cultures from which the cloudiness had disappeared were considered positive. Smears were made on glass slides from these positive cultures. A Neufeld "Quellungs" reaction was carried out on these smears.

The Gargle Method.--The individual was asked to rinse out his mouth with 25 cc. of sterile physiological saline solution (0.85 per cent) and to discard this washing. Another 25 cc. of sterile physiological saline solution was used immediately as a gargle for 1 to 3 minutes. This liquid was expelled into a sterile container and then centrifuged at high speed (approximately 2000 r.p.m.) for 45 minutes. The supernatant liquid was poured off and the sediment suspended in 1 cc. of sterile salt solution. One cc. of this suspension was injected intraperitoneally into a white mouse. The method from here was the same as that for the swab method, i.e., withdrawal after 6 hours, solubility test, and Neufeld typing.

As soon as possible after an inoculated mouse died, an autopsy was made. By means of a small capillary pipette blood was withdrawn from the heart. A drop of this blood was placed in enriched beef heart infusion broth and incubated at 37° C. until noticeable cloudiness developed. The culture was then tested for solubility using sodium desoxycholate and the

Neufeld reaction was carried out as for the others.

After 14 to 15 days the mice which did not die were chloroformed and cultures taken from the heart's blood. If growth occurred it was taken through the regular procedure.

The solubility test using sodium desoxycholate solution and the phenomenon of capsular swelling described by Neufeld (1902) and later used by Neufeld and Etinger-Tulczynska (1933) as a method for rapid type determination of Diplococcus pneumoniae, are the ones used at the present time by the author for the identification of types of diplococci. For this Neufeld "Quellungs" reaction, antisera of types 1 to 32, according to Cooper (1929), and antiserum for type 33, are placed into groups A, B, C, D, E, and F. Each group serum contains the pooled antisera for certain types of pneumococci.

The method for carrying out the Neufeld "Quellungs" reaction in this survey was as follows: Two smears of the soluble cultures were placed on each of six clean glass slides. A different group antiserum, obtained from the Lederle Laboratories, was placed on each smear. In the group antisera used there was a blue dye which stained the organisms. The smear was then covered with a clean cover glass and was examined microscopically with the aid of the oil immersion lens. A positive reaction was indicated by a clear, unstained area around the stained pneumococci. This area has a definite outline and it is the sharpness of this outline which is more important in type determination than the amount of swelling of the capsule. Halos may be seen in negative preparations, due, probably, to the presence of a small capsule around the organism or to the re-

fraction of light when an object is out of focus. On changing the focal plane, these halos will disappear, but in positive cultures they will still be visible. When the positive group was determined, a loopful of a different type antiserum (of the types included in the positive group) was placed on each of the remaining smears and a loopful of methylene blue was added to stain the organisms. A clean cover glass was placed on the smear, which was examined as described previously. All smears were carefully examined microscopically to determine whether more than one type of pneumococcus was present. All negative slides were set aside for approximately one hour and then checked and rechecked.

RESULTS

Number of individuals who were carriers (tables I and I-a).--Of the 54 individuals included in this survey, from whom a total of 699 cultures were taken by the two methods, 41 (75.9 per cent) were carriers of *Diplococcus pneumoniae* at one time or another.

Of a total of 351 survey tests (each test consisting of the swab and the gargle made from one individual on one day), 140 (39.8 per cent) gave positive cultures.

Number of carriers obtained by each method (tables II and II-a).--Ninety-two (65.7 per cent) of the 140 positive cultures were obtained by the gargle method; 97 (69.3 per cent) by the swab method; and 49 (35.0 per cent) were obtained with both methods from the same individual on the same day.

Occurrence of positive cultures by the month (tables I and I-a).--

TABLE I

THE INCIDENCE OF PNEUMOCOCCI IN THE THROATS OF A GROUP OF

INDIVIDUALS FROM MONTANA STATE COLLEGE
(1940-1941)

Individual No.	October	November	December	January	February	March	April
1	-	-	-	-	-	-	-
2	-	-	-	+	-	+	+
3	-	-	-	-	+	+	+
4	+	+	-	+	-	-	-
5	+	+	+	-	-	not tested	-
6	+	-	-	+	+	-	-
7	+	-	-	+	-	+	-
8	+	+	+	+	+	+	not tested
9	+	+	+	+	+	+	+
10	+	-	-	Dropped school.....			
11	+	-	-	-	-	-	-
12	+	not tested	+	+	+	+	-
13	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-
17	-	-	-	-	-	-	not tested
18	-	-	-	+	-	+	not tested
19	-	-	+	-	-	-	-
20	Dropped school.....						
21	+	+	+	not tested	+	-	-
22	-	-	-	-	-	-	-
23	+	+	+	-	-	-	+
24	+	-	-	+	+	-	not tested
25	+	+	+	+	+	-	not tested
26	-	-	-	-	-	+	-
27	-	-	-	+	+	+	+

TABLE I (concld.)

Individual No.	October	November	December	January	February	March	April
28	-	-	-	-	-	-	-
29	+	+	-	+	-	+	-
30	-	-	-	-	-	-	not tested
31	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-
33	+	+	+	-	-	+	-
34	+	+	-	-	-	-	-
35	+	+	+	+	-	+	-
36	+	+	+	+	+	+	+
37	+	+	+	-	-	-	not tested
38	-	+	-	-	-	-	-
39	+	-	not tested	not tested	-	-	not tested
40	+	not tested	+	+	+	not tested	-
41	-	-	-	-	-	not tested	+
42	-	-	-	-	+	-	+
43	+	+	+	-	-	+	-
44	+	-	-	not tested	-	-	+
45	+	+	+	+	+	+	+
46	+	+	+	-	-	-	-
47	-	-	-	+	-	-	+
48	-	-	+	+	+	+	+
49	-	-	-	+	+	+	not tested
50	-	+	+	-	-	not tested	+
51	-	-	-	-	-	-	-
52	+	+	+	+	+	+	not tested
53	+	not tested	-	-	-	+	+
54	-	-	-	+	-	-	-
55	not tested	-	-	-	-	-	not tested

+ means that the individual gave a positive test.
- means that the individual gave a negative test.

TABLE I-a

SUMMARY OF THE OCCURRENCE OF PNEUMOCOCCI
October 1940 to March 1941

Month	Number persons positive	Number persons tested	Per cent positive
October	27	53	50.9
November	18	51	35.5
December	19	53	35.9
January	21	50	42.0
February	16	53	30.2
March	19	49	38.8
April	14	42	33.3
Total	140	351	39.8

Total number persons used in the survey.....54

Number persons giving positive cultures at one time or another.....41

Per cent of persons showing pneumococci at one time or another.....75.9

Total number of swab and gargle cultures taken during the survey.....699

TABLE II

A COMPARISON OF THE GARGLE AND THE SWAB TECHNICS IN THE ISOLATION OF

PNEUMOCOCCI FROM THE THROATS OF A GROUP OF INDIVIDUALS AT MONTANA STATE COLLEGE
(1940-1941)

Individual No.	October		November		December		January		February		March		April	
	G	S	G	S	G	S	G	S	G	S	G	S	G	S
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	+	+	-	-	-	+	-	+
3	-	-	-	-	-	-	-	-	+	-	+	-	+	+
4	-	+	+	-	-	-	-	+	-	-	-	-	-	-
5	+	-	+	-	+	-	-	-	-	-	not tested		-	-
6	-	+	-	-	-	-	-	+	+	-	-	-	-	-
7	+	+	-	-	-	-	-	+	-	-	+	-	-	-
8	-	+	+	+	-	+	+	+	-	+	-	+	not tested	
9	+	-	-	-	+	+	+	+	-	+	-	+	+	-
10	+	-	-	-	-	-	Dropped school.....							
11	-	+	-	-	-	-	-	-	-	-	-	-	-	-
12	-	+	not tested		-	+	-	+	+	+	+	+	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-	not tested	
18	-	-	-	-	-	-	+	+	-	-	-	+	not tested	
19	-	-	-	-	-	+	-	-	-	-	-	-	-	-
20	Dropped school.....													
21	+	+	-	+	+	+	not tested		+	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	+	+	+	-	+	-	-	-	-	-	-	-	+	-
24	+	+	-	-	-	-	+	+	+	-	-	-	not tested	
25	+	+	+	+	+	-	-	+	+	-	-	-	not tested	
26	-	-	-	-	-	-	-	-	-	-	+	+	not tested	-

TABLE II (concl.)

Individual No.	October		November		December		January		February		March		April	
	G	S	G	S	G	S	G	S	G	S	G	S	G	S
27	-	-	-	-	-	-	+	+	+	+	+	+	+	+
28	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	-	+	-	+	-	-	+	+	-	-	-	+	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-	not tested	
31	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	+	+	+	-	-	+	-	-	-	-	+	-	-	-
34	-	+	-	+	-	-	-	-	-	-	-	-	-	-
35	+	+	-	+	+	-	+	+	-	-	+	+	-	-
36	+	+	+	+	+	+	+	+	+	-	+	+	-	+
37	-	+	+	-	+	-	-	-	not tested	-	-	-	not tested	
38	-	-	-	+	-	-	-	-		-	-	-	-	-
39	+	-	-	-	not tested		not tested		-	-	-	-	not tested	
40	+	+	not tested		-	+	+	+	+	-	not tested		-	-
41	-	-	-	-	-	-	-	-	-	-	not tested		-	+
42	-	-	-	-	-	-	-	-	+	+	-	-	+	-
43	+	+	+	+	+	-	-	-	-	-	+	-	-	-
44	-	+	-	-	-	-	not tested		-	-	-	-	+	-
45	+	+	+	+	+	+	+	+	-	+	+	+	-	+
46	+	+	+	+	+	-	-	-	-	-	-	-	-	-
47	-	-	-	-	-	-	+	-	-	-	-	-	-	+
48	-	-	-	-	+	+	-	+	+	-	-	+	+	-
49	-	-	-	-	-	-	+	+	-	+	+	-	not tested	
50	-	-	-	+	+	+	-	-	-	-	not tested		+	+
51	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52	+	+	+	+	+	+	+	+	+	+	+	+	not tested	
53	+	-	not tested		-	-	-	-	-	-	+	+	+	-
54	-	-	-	-	-	-	-	+	-	not tested	-	-	-	-
55	not tested		-	-	-	-	-	-	-		-	-	not tested	

G means Gargle method was used.
S means Swab method was used.

\- means no pneumococci were found.
\+ means pneumococci were found.

TABLE II-a

A COMPARISON OF THE RELATIVE MERITS OF THE GARGLE AND THE SWAB METHODS FOR

THE ISOLATION OF DIPLOCOCCUS PNEUMONIAE
(1940 - 1941)

Month	Number persons giving positive cultures: with Gargle	with Swab	with Both	Total persons positive	Per cent of total positives which each method gave: with Gargle	with Swab	with Both	Number persons tested	Per cent of total persons tested which were positive: with Gargle	with Swab	with Both
October	18	22	13	27	66.6	81.5	48.1	53	33.9	41.5	24.5
November	12	13	7	18	66.7	72.2	38.2	51	23.5	25.5	13.7
December	14	12	7	19	73.7	63.2	36.8	53	26.4	22.6	13.2
January	14	20	7	27	66.7	74.1	33.3	50	28.0	40.0	14.0
February	12	8	4	16	75.0	50.0	25.0	53	22.6	15.1	7.5
March	13	14	8	19	68.4	73.7	42.1	49	26.5	28.6	16.3
April	9	8	3	14	64.3	57.1	21.4	42	21.4	19.0	7.1
Total	92	97	49	140	65.7	69.3	35.0				

By studying table I-a the reader will note that during the period of seven months from October 1940 to April 1941 the per cent of positive reactions varied from 30.2 to 50.9. The lowest per cent occurred during the month of February, the highest in October.

Occurrence of cultures which did not give a positive Neufeld reaction (tables III and III-a).--Frequently it was found that a culture, apparently a pneumococcus, which was soluble or partially soluble when tested with sodium desoxycholate solution failed to give a positive Neufeld "Quellungs" reaction. From table III-a it can be seen that the number of these cultures varied from 14 in October and December to 3 in March.

Kinds of carriers and the number of persons in each classification (tables IV and IV-a).--After having studied the division of carriers as made by Webster and Hughes (1931) and Bliss, McClaskey, and Long (1934), the present investigator used the following terms and definitions for the purpose of classifying the pneumonia carriers:

A non-carrier is a person who never gives a positive culture of Diplococcus pneumoniae.

A chronic carrier is one who yields positive cultures of the same type of pneumococci for three or more consecutive months.

An intermittent carrier is one who yields positive cultures at intervals during the survey. The cultures, when of the same type, occur not more than two consecutive months.

A temporary carrier is one who yields only one positive culture during the survey.

TABLE III

OCCURRENCE OF SOLUBLE CULTURES WHICH DID NOT

GIVE POSITIVE NEUFELD REACTIONS
(1940-1941)

Individual No.	October	November	December	January	February	March	April
1	p. sol.	-	-	-	-	-	-
2	sol.	sol.	-	-	-	-	-
3	-	-	sol.	sol.	-	-	-
4	-	-	p. sol.	-	-	-	-
5	-	-	-	-	-	not tested	sol.
6	-	-	-	-	-	-	p. sol.
7	-	p. sol.	sol.	-	p. sol.	-	-
8	-	-	-	-	-	-	not tested
9	-	-	-	-	-	-	-
10	-	p. sol.	-	Dropped school.....			
11	-	-	-	p. sol.	-	-	-
12	-	not tested	-	-	-	-	-
13	p. sol.	-	p. sol.	p. sol.	-	-	-
14	p. sol.	-	-	-	-	-	sol.
15	p. sol.	p. sol.	p. sol.	-	p. sol.	-	-
16	-	-	-	-	-	-	-
17	-	-	-	-	-	-	not tested
18	p. sol.	-	-	-	p. sol.	-	not tested
19	-	-	-	-	-	-	-
20	Dropped school.....						
21	-	-	-	not tested	-	-	-
22	-	-	-	-	p. sol.	-	-
23	-	-	-	-	-	-	-
24	-	-	sol.	-	-	-	not tested
25	-	-	-	-	-	-	not tested
26	-	-	-	-	-	-	-
27	sol.	-	-	-	-	-	-

TABLE III (concl.)

Individual No.	October	November	December	January	February	March	April
28	-	-	-	-	p. sol.	sol.	-
29	-	-	sol.	-	-	-	-
30	-	-	-	-	-	-	not tested
31	-	-	sol.	-	-	-	-
32	-	-	sol.	-	sol.	sol.	-
33	p. sol.	sol.	sol.	sol.	-	-	-
34	-	p. sol.	p. sol.	p. sol.	-	-	-
35	-	-	-	-	-	-	sol.
36	-	-	-	-	-	-	-
37	-	-	-	-	-	-	not tested
38	sol.	-	-	-	-	-	-
39	p. sol.	-	not tested	not tested	-	-	not tested
40	-	not tested	-	-	-	not tested	-
41	-	p. sol.	-	-	-	not tested	-
42	sol.	-	sol.	-	-	-	-
43	-	sol.	-	p. sol.	sol.	-	-
44	-	-	p. sol.	not tested	-	-	-
45	-	-	-	-	-	-	-
46	-	-	-	-	-	sol.	-
47	-	-	-	-	-	-	-
48	sol.	sol.	-	-	-	-	-
49	p. sol.	-	-	-	-	-	not tested
50	-	-	-	-	p. sol.	not tested	-
51	sol.	sol.	-	-	-	-	sol.
52	-	-	-	-	-	-	not tested
53	-	not tested	sol.	-	-	-	-
54	-	-	-	-	-	-	-
55	not tested	sol.	-	p. sol.	-	-	not tested

sol. means that the culture was completely soluble with sodium desoxycholate solution.
p. sol. means that the culture was partially soluble with sodium desoxycholate solution.

TABLE III-a

SUMMARY OF THE "SUSPICIOUS" AND THE POSITIVE CULTURES
(October 1940-April 1941)

	October	November	December	January	February	March	April
Total "suspicious" cultures	14	11	14	7	7	3	5
Total positive cultures	27	18	19	21	16	19	14
Total	41	29	33	28	23	22	19
Total persons tested/month	53	51	53	50	53	49	42
Gross per cent "positive"	77.4	56.9	62.3	56.0	43.3	45.0	45.2

TABLE IV

THE INCIDENCE OF THE SPECIFIC TYPES OF PNEUMOCOCCI IN THE THROATS OF

A GROUP OF INDIVIDUALS AT MONTANA STATE COLLEGE
(1940-1941)

Individual No.	October	November	December	January	February	March	April
1	-	-	-	-	-	-	-
2	-	-	-	15	-	15	15
3	-	-	-	-	9	8	8,13
4	5	3	-	3	-	-	-
5	25	25	17	-	-	not tested	-
6	25	-	-	28	28	-	-
7	17,24	-	-	15	-	3	-
8	6	8,9	8	4,8	9	8	not tested
9	3	3	3	3	3	3	3
10	19	-	-	Dropped school			
11	3	-	-	-	-	-	-
12	4	not tested	20	20	20	20	-
13	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-
17	-	-	-	-	-	-	not tested
18	-	-	-	4	-	4,15	not tested
19	-	-	3	-	-	-	-
20	Dropped school						
21	17	17	17	not tested	17	-	-
22	-	-	-	-	-	-	-
23	18	18	16	-	-	-	18
24	17	-	-	17	17	-	not tested
25	8	8	8	8	8	-	not tested
26	-	-	-	-	-	4,6	-
27	-	-	-	3	3	3	3

TABLE IV (concl.)

Individual No.	October	November	December	January	February	March	April
28	-	-	-	-	-	-	-
29	6	6	-	15	-	24	-
30	-	-	-	-	-	-	not tested
31	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-
33	24	3	B	-	-	3	-
34	28	28	-	-	-	-	-
35	3,6	3	3	3	-	3	-
36	15	13	13	13	13	13	13
37	28	28	28	-	-	-	not tested
38	-	3	-	-	-	-	-
39	28	-	not tested	not tested	-	-	not tested
40	20	not tested	20	3	3	not tested	-
41	-	-	-	-	-	not tested	17
42	-	-	-	-	32	-	32
43	19,28	E	E	-	-	E	-
44	31	-	-	not tested	-	-	8
45	17	17	17	17	17	17	17
46	31	31	31	-	-	-	-
47	-	-	-	8	-	-	32
48	-	-	6	6	6	6	6
49	-	-	-	4,8	4	4	not tested
50	-	3	3	-	-	not tested	12
51	-	-	-	-	-	-	-
52	6,28	6	6	6,19	19	19	not tested
53	6	not tested	-	-	-	4	4
54	-	-	-	4	-	-	-
55	not tested	-	-	-	-	-	not tested

B Means Group B
E Means Group E

TABLE IV-a

A COMPARISON OF THE GARGLE AND THE SWAB TECHNICS IN THE ISOLATION OF THE SPECIFIC TYPES OF PNEUMOCOCCI IN THE THROATS OF INDIVIDUALS AT MONTANA STATE COLLEGE (1940-1941)

Individual No.	October		November		December		January		February		March		April	
	G	S	G	S	G	S	G	S	G	S	G	S	G	S
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	15	15	-	-	-	15	-	15
3	-	-	-	-	-	-	-	-	9	-	8	-	8,13	8
4	-	5	3	-	-	-	-	3	-	-	-	-	-	-
5	25	-	25	-	17	-	-	-	-	-	not tested		-	-
6	-	25	-	-	-	-	-	28	28	-	-	-	-	-
7	24	17	-	-	-	-	-	15	-	-	3	-	-	-
8	-	6	9,8	8	-	8	4	8	-	9	-	8	not tested	
9	3	-	-	-	3	3	3	3	-	3	-	3	3	-
10	19	-	-	-	-	-	Dropped school.....							
11	-	3	-	-	-	-	-	-	-	-	-	-	-	-
12	-	4	not tested		-	20	-	20	20	20	20	20	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-	not tested	
18	-	-	-	-	-	-	4	4	-	-	-	4,15	not tested	
19	-	-	-	-	-	3	-	-	-	-	-	-	-	-
20	Dropped school.....													
21	17	17	-	17	17	17	not tested		17	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	18	18	18	-	16	-	-	-	-	-	-	-	18	-
24	17	17	-	-	-	-	17	17	17	-	-	-	not tested	
25	8	8	8	8	8	-	-	8	8	-	-	-	not tested	
26	-	-	-	-	-	-	-	-	-	-	4	6	not tested	-

TABLE IV-a (concl.)

Individual No.	October		November		December		January		February		March		April	
	G	S	G	S	G	S	G	S	G	S	G	S	G	S
27	-	-	-	-	-	-	3	3	3	3	3	3	3	3
28	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	-	6	-	6	-	-	15	15	-	-	-	24	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-	not tested	
31	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	24	24	3	-	-	B	-	-	-	-	3	-	-	-
34	-	28	-	28	-	-	-	-	-	-	-	-	-	-
35	3	6,3	-	3	3	-	3	3	-	-	3	3	-	-
36	13	13	13	13	13	13	13	13	13	-	13	13	-	13
37	-	28	28	-	28	-	-	-	-	-	-	-	not tested	
38	-	-	-	3	-	-	-	-	-	-	-	-	-	-
39	28	-	-	-	not tested		not tested		-	-	-	-	not tested	
40	20	20	not tested		-	20	3	3	3	-	not tested		-	-
41	-	-	-	-	-	-	-	-	-	-	not tested		-	17
42	-	-	-	-	-	-	-	-	32	32	-	-	32	-
43	28,19	19	E	E	E	-	-	-	-	-	E	-	-	-
44	-	31	-	-	-	-	not tested		-	-	-	-	8	-
45	17	17	17	17	17	17	17	17	-	17	17	17	-	17
46	31	31	31	31	31	-	-	-	-	-	-	-	-	-
47	-	-	-	-	-	-	8	-	-	-	-	-	-	32
48	-	-	-	-	6	6	-	6	6	-	-	6	6	-
49	-	-	-	-	-	-	8	4,8	-	4	4	-	not tested	
50	-	-	-	3	3	3	-	-	-	-	not tested		12	12
51	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52	28	6	6	6	6	6	6	19	19	19	19	19	not tested	
53	6	-	not tested		-	-	-	-	-	-	4	4	4	-
54	-	-	-	-	-	-	-	4	-	not tested	-	-	-	-
55	not tested		-	-	-	-	-	-	-	-	-	-	not tested	

G means the Gargle method was used.
S means the Swab method was used.
B means Group B.
E means Group E.

A multiple-type carrier is one who carries two or more types simultaneously.

Using the author's classification, of the 54 individuals included in this survey 12 (22.2 per cent) were non-carriers of Diplococcus pneumoniae; 14 (25.9 per cent) were chronic carriers; 27 (50.0 per cent) were intermittent carriers; 5 (9.2 per cent) were temporary carriers; and 9 (16.6 per cent) were carriers of more than one type during one survey.

Frequency of occurrence of specific Diplococcus pneumoniae types (table V).--During this survey, type 3 was found 37 times; type 17, 26 times; type 6, 18 times; type 8, 18 times; type 4, 13 times; type 13, 13 times; and type 28, 10 times. The other types which occurred less than 10 times during the survey were 20, 19, 15, 31, 24, 18, 32, 25, 9, 16, 5, and 12. Types 1 and 2 were never found in this work.

DISCUSSION OF METHODS AND RESULTS

The methods of obtaining cultures used in this survey. a) Why two were chosen.--A survey was carried out by the author during November 1939 to April 1940 on a small group of individuals at Montana State College. After two months, when the results of the survey indicated that the carrier rate was far below that found by such investigators as Powell, Atwater, and Felton (1926) (table VI) the author wrote to investigators who had carried out similar studies in this field.

Smillie (1940) reported that he obtained the best results by taking a culture from the posterior nasopharynx, care being taken not to touch

TABLE V

THE SPECIFIC TYPES OF PNEUMOCOCCI ARRANGED IN THE ORDER OF THEIR FREQUENCY DURING OCTOBER 1940 - MARCH 1941

Types of Pneumococci	Number of times found
3	37
17	26
6	18
8	18
4	13
13	13
28	10
20	9
19	8
15	8
31	6
24	4
18	4
32	4
25	3
9	3
16	1
5	1
12	1

TABLE VI

A SUMMARY OF THE OCCURRENCE OF PNEUMOCOCCI
DURING NOVEMBER 1939 to APRIL 1940/

Month		Number persons positive	Number persons tested	Per cent positive
November	(s)*	1	26	3.8
December	(s)	2	26	7.7
January	(g)#	6	33	18.3
February	(g)	4	34	11.8
March	(g)	3	31	9.7
April	(g)	7	33	21.2
Total		23	187	12.3

*Swab method used.
#Gargle method used.

/ Due to the lack of experience on the part of the investigator and to the lack of uniformity in the methods used to obtain cultures, these figures should not be considered indicative of the exact number of carriers of Diplococcus pneumoniae. They are, however, suggestive.

any part of the mouth cavity. He stated that he would expect at least 40 to 50 per cent of normal people to carry pneumococci in the summer, and in winter, especially around the first of March, the proportion should be 70 per cent.

Felton (1940) reported that the swab method was a poor technic for isolating pneumococci from carriers or normal individuals. He suggested the use of the gargle technic which was described earlier in this paper.

There seemed to be a divergence of opinion as to the relative merits of the two methods mentioned and, in some work carried out by the author from November 1939 through April 1940 (table VI), there appeared to be a noticeable variation in the results obtained by each method. Hence it was decided that a comparison of the gargle and the swab methods might be of value.

As a result, beginning with October 1940, a group of 54 individuals at the Montana State College campus was selected to be cultured each month. This group included students from various departments, some members of the college faculty, and some members of the clerical staff. It was hoped that by taking individuals at random, the results obtained might approximate the carrier situation as it existed on the campus.

b) The advantages and the disadvantages of each method of obtaining cultures.--At best, each of the two methods used had several disadvantages. In the following discussion, the most outstanding advantages and disadvantages will be mentioned.

The first method used on each individual was that of the swab. A flat, wooden, tongue depressor was placed on the back of the tongue to aid

in getting the swab into the posterior nasopharynx with as little chance of touching the interior of the mouth as possible. It was at this point that the first major difficulty arose. Some individuals would constrict their throats so tightly that it was almost impossible to get the swab very high into the posterior nasopharynx. Equally important in hindering the most effective use of the swab was the gagging which occurred in a large number of the individuals.

The second difficulty arose when the swab was in place. It was possible with some individuals, to rub the walls of the posterior nasopharynx with the swab. With others the degree of rubbing was much less, the result being that the surface covered by the swab was appreciably less in some than in others. This failure to cover as much surface as desired with the swab might mean the difference between a positive and a negative result. If the organisms tended to be localized in the nasopharynx, it is quite conceivable that the swab might have missed them.

The third difficulty was that during the period of incubation preceding centrifugation, there might have been, in some cases, an overgrowth of the pneumococci by some other organisms. However, it seems to the author that the probability of this is very small. Due to the "lag" period which follows the introduction of organisms into a culture medium, it is quite likely that the danger of overgrowth by any intruding organism is negligible when the period of incubation is only 6 hours at 37° C.

In considering the advantages of the swab method, there are two which seem worthy of mention.

The first advantage is that, although there will be some variations

in the amount of rubbing and in the distance that the swab enters into the nasopharynx, the method can be more nearly standardized by the investigator than can the gargle method.

The second advantage is that organisms which are present in the posterior nasopharyngial region and which may not be present in the back of the mouth may be obtained by the use of the swab method.

After the individual had been swabbed, he was asked to rinse his mouth with sterile physiological saline solution. The purpose of this rinse was to reduce the number of intruding microorganisms.

Each individual was asked to gargle from 1 to 3 minutes, but very seldom did anyone gargle longer than 1½ minutes. Some individuals claimed they were unable to gargle. As a result of this belief, they would not try to gargle longer than 30 seconds. In other words, one of the greatest disadvantages of this method was the variability in the length of time each individual gargled.

Another disadvantage of this method was that each individual gargled in a different way from any other person. In other words, if an individual gargled with his throat tense, the salt solution would neither be able to penetrate the cracks and crevices of the throat nor to come in contact with the back part of the throat. Therefore it seems to the author that, assuming microorganisms to be present, the persons gargling with a relaxed throat would be much more apt to give a positive culture than those with a tense throat.

Throughout this survey, it was the aim of the author to call in the individuals to be cultured preceding a meal rather than immediately

following a meal. It was felt that after an individual had eaten, the chances of getting pneumococcus organisms would be materially lessened. However, since the time when the individuals could come in was governed to a great extent by their schedule of classes, it was not always possible to culture them just previous to a meal.

It was found that when some individuals gargled, the amount of liquid left from the gargling was reduced from 25 cc. to 10 cc. This, of course, meant that the individual had swallowed over half of the saline solution. Since the number of pneumococci in a sample probably is very small, it is readily seen that the less the amount remaining, the less chance there would be for getting the organisms. This factor is probably not as important as the first three mentioned. However, it was believed that since it was one of the variables in the survey it should be mentioned.

The possible reasons for positive cultures being obtained by one method and not by the other in the case of multiple-type carriers.-- It was not clear in the papers of some investigators whether, when they mentioned multiple-type carriers, they meant persons who harbored several different types of Diplococcus pneumoniae in the throat at one time or another during the survey or whether they meant persons who harbored more than one type in the throat at one time. In this paper, when the author uses the term "multiple-type carriers", the reference will be to persons who harbor more than one specific type of Diplococcus pneumoniae in their throats at one time.

From table IV-a it can be seen that individuals 3, 7, 8, 18, 26,

35, 43, 49, and 52 had two specific types of pneumococci in their throats in the same survey. On closer inspection of the tables, it is noticed that both types were not always found at the same time with the same method. For example, in the case of individual 7, type 24 was isolated by means of the gargle method and type 17 by means of the swab method in the October survey.

An explanation of such results might lie in the location of the organisms. If type 17 organisms were localized in the posterior nasopharynx, behind the soft palate, it is quite conceivable that the swab method would be the only one to reach the organisms.

Likewise, if type 24 organisms were located on or around the tonsilar crypts, the gargle method would be the one most likely to obtain these organisms--especially since it was the aim to keep the swab from touching the mouth.

The question naturally arises as to whether there might not be members of type 17 organisms in the mouth and members of type 24 in the posterior nasopharynx.

Since Diplococcus pneumoniae is not a motile organism, it would have to rely on artificial methods of transfer. As the posterior nasopharynx is not in the direct path of liquids, food, or saliva which are swallowed, the chance of organisms from the mouth being transported to that region seems rather slight.

The chance of organisms being transported from the posterior nasopharynx to the mouth cavity would probably be greater than from the mouth to the nasopharynx for the reason that the secretions pass down-

ward more easily than upward.

There is a possibility that if the method of "blocking" which Bliss, McClaskey, and Long (1934) found to be successful had been carried out the results might have been different. These authors found that by injecting two mice for each individual, one with a throat swab broth culture alone and the other with a mixture of 0.3 cc. of culture and 0.3 cc. of immune serum of the type of the predominant pneumococcus, they could ascertain the predominant type, block its lethal effect with antiserum, and reveal a subordinate type.

In those cases where more than one type was found during the survey, as in individuals 3, 4, 5, 7, 8, 12, 18, 23, 29, 33, 35, 40, 43, 49, and 52, had the method of "blocking" been used, it might have been possible to have shown the presence of a type which was submerged.

Possible reasons for cultures not typing out.--Throughout the course of this survey there were quite a number of cultures which failed to give a typical Neufeld "Quellungs" reaction. When a culture showed cloudiness, a solubility test was carried out using a 10 per cent solution of the sodium desoxycholate (as described in the section on methods). Some of the soluble or partially soluble cultures failed to type out (table III). Naturally, reasons for such a phenomenon were sought.

One of the first possibilities was that the slides were not thoroughly checked, but this could not apply here since all slides were carefully examined and all negative slides were set aside for $\frac{1}{2}$ hour to one hour and then checked again. Frequently two persons checked the slides before placing them in the distinctly negative group.

The second possibility was that the number of pneumococci was so small that they would be overlooked when examining the slides. It would seem that if the number of organisms was sufficiently great to show a noticeable to a complete clearing when sodium desoxycholate solution was added, the organisms would be present in numbers great enough to be found.

The third, and most probable explanation, is that there were present members of the Diplococcus pneumoniae group for which there was no specific antiserum.

Kauffmann, Mørch, and Schmith (1940) in the course of the past two or three years have determined serologically 20 new types of pneumococci, so that there is at present a total of 50 different serological types known. They say:

> Contrary to the widespread idea that the diagnosis of pneumococcus types with the Neufeld reaction is a very simple method, our view is that the appraisal of this reaction is often difficult and that it depends on the selection and adaptation of the sera employed. In most cases, in order to make a reliable pneumococcus type-diagnosis it is necessary to employ certain absorbed immune sera, as in some cases in practice one must make diagnosis twice, i.e., first establish the fact that the type belongs for example to the 7 group, and then by means of other absorbed sera make the more exact type-diagnosis.

Assuming that these soluble and partially soluble cultures were unidentified types of pneumococci, the percentage of individuals who yielded positive cultures by the month would more nearly approximate the results of other investigators (table III-a).

It has been found by Walter, Blount, Beattie, and Cotler (1940) that type 33 is relatively avirulent for mice and that strains of this type sometimes require the use of partially anaerobic conditions for successful cultivation when first isolated from an individual. Since anaerobi-

osis was never used in this survey, it may be possible that cultures of type 33 pneumococci were missed.

The importance of contacts in this survey.--Unless a survey is carried out in a manner different from this it is almost impossible to determine the importance of carrier contacts. In the case of the 14 sorority sisters, there are a few interesting observations to be made.

Type 3 appeared in October in individual 9 and in November in her roommate, individual 4. Individuals 3 and 8 were affectionate roommates which perhaps accounts for the fact that for two months (February and March) both carried the same type of organisms. Individuals 7 and 9 were frequently together which might possibly explain the occurrence of type 3 organisms in both at the time of the March survey. However, in other individuals where the same type appeared there was no known direct contact which would adequately explain the similarity.

The possibility of one distinct type of Diplococcus pneumoniae changing into another distinct type.--Throughout the past few years there has been one question which perplexed many investigators. It was this: "Does one specific type of Diplococcus pneumoniae change into another specific type under natural conditions?" From the work of Megrail and Ecker (1924), Barnes and Wright (1936) and Dawson (1930) there seems to be no conclusive evidence that such a transformation occurs. Therefore, in this paper, the possibility of such a change has been disregarded.

Comparison of results obtained by the author with those of other investigators.--During the seven month's survey carried out by the writer, a total of 351 persons were tested. Of these 39.8 per cent (140) were

found to be carriers of Diplococcus pneumoniae.

These results were lower than those of Powell, Atwater, and Felton (1926) whose total results were as follows:

a) Of 104 laboratory workers, 67 per cent were carriers.

b) Of 112 high school boys, 66 per cent were carriers.

c) Of 112 medical school students, 66 per cent were carriers.

d) Of 90 student nurses, 53 per cent were carriers.

This difference might be explained partly by the fact that these investigators obtained their results in Boston where the contacts of the individuals were probably closer than in Montana.

The difference might also be due to the choice of organisms to be called positive. Powell, Atwater, and Felton (1926) say:

> We have included as pneumococci such Gram-positive, encapsulated diplococci as were bile soluble, capable of fermenting inulin and which, in the case of Types I, II, and III, agglutinated specifically, but, in the case of Type IV, did not agglutinate with type sera.

The author did not use the agglutination test but used instead the solubility test and the Neufeld "Quellungs" reaction. Only those organisms which gave a definite Neufeld reaction were included as positive. It is thought that if typing sera for the types which Kauffmann, Mørch, and Schmith (1940) reported had been used, the results might have been higher.

Blacklock and Guthrie (1933) found pneumococci in the throats of 38.6 per cent of the healthy children they examined. This percentage is quite noticeably lower than that of the author who found 75.9 per cent of the 54 individuals included in the survey to be carriers. This

difference might be explained by the fact that the former investigators used only the swab method to obtain cultures from the throat. Lack of information concerning the number of children examined and the procedures used by Blacklock and Guthrie makes one hesitate to draw too many conclusions as to the reasons for the difference in results.

Schleifstein (1938), investigating 100 normal individuals, found 73 per cent to be carriers. His method was to swab the nasopharynx and both tonsils. In this way he obtained organisms from the same regions as did the writer when the gargle and the swab methods were used. He also identified his organisms by using the bile solubility test and the Neufeld reaction. It is gratifying to note that, although his group of individuals was larger, his results are very comparable with those of the author. This might indicate that, had other investigators used methods which were more alike, the results would have been less different.

Webster and Hughes (1931) found 80 per cent of 105 children and adults in New York to be carriers. These results were 6 per cent higher than those of the author, but it may be that the differences in locality as well as the differences in methods account for this slight variation.

Bliss, McClaskey, and Long (1934) made a study of 20 subjects on whom 1,016 cultures were taken over a period of two years. All but one of the subjects yielded positive cultures of Diplococcus pneumoniae at least once during the course of the investigation. They obtained their cultures by wiping the posterior pharynx and the tonsils or tonsilar beds with cotton swabs. Needless to say, this percentage is much higher than the one obtained in this survey. Since there does seem to be a notice-

able fluctuation in the prevalence of carriers, it is not at all improbable that, had the present author carried out an investigation embracing a two year period, the results might have more nearly approximated those of the former investigators.

Rosenau, Felton, and Atwater (1926) reported that Sailer, Hall, Wilson, and McCoy (1917) found 16.0 per cent of 700 examinations made to be positive. To make a comparison possible, the results of the present survey must be figured on the same basis. Of 699 examinations made, the author found 27.0 per cent (189) to be positive. These results are slightly higher than the former ones given.

In a five month's survey on 24 medical school students, Powell, Atwater, and Felton (1926) found the highest incidence of pneumococci to be in October (83 per cent) and the lowest to be in November (42 per cent). The other months were in the following order in respect to the occurrence of pneumococci in the throat: February, January, and December. These percentages were very much higher than those in the present survey. The group tested by the author was almost twice as large and was made up of a more heterogenous group of people. The highest incidence found by this investigator was in the month of October (50.9 per cent) and the lowest was in February (30.2 per cent) with the other months in the following order: January, March, December, November, and April (table I-a). Here again, the differences in the locality and in the methods used may explain the lack of similarity in the results.

Variable factors which may alter the results with surveys of this kind.--The first factor which may influence the results is the method

used. It is extremely difficult and highly unsatisfactory to try to compare the results obtained by the swab-culture-mouse method with those obtained by the gargle-culture-centrifuge-mouse method. As has been pointed out previously, the way the culture is taken, whether swab or gargle, may alter the results.

The second factor is the kind of culture medium used. Some found Avery's broth quite satisfactory. Others used blood agar plates, and still others used beef infusion broth. The author used buffered beef heart infusion broth during the survey from November 1939 to April 1940 (table VI). In the survey made from October 1940 to April 1941, buffered beef heart infusion broth enriched with beef serum was used. To get an approximate idea of the effect of adding serum, the author used unenriched beef heart infusion broth during the November 1940 survey along with the enriched broth. The results were startling. Twenty-seven out of 53 persons were positive when enriched broth was used and only 10 out of the 53 were positive when the unenriched broth was used. These results, although carried out on only a very small number, seem to indicate that a medium enriched with serum will give a much better growth of pneumococci than will an unenriched medium.

A third factor which may influence the results is the possibility of missing positive cultures. It has already been suggested that types above 33, for which there were no typing sera available to the author, may have been present and consequently missed. There is also a possibility that avirulent forms of Diplococcus pneumoniae may have been present in numbers small enough to have been successfully resisted by

the mouse or to have been killed by antagonism from the presence of some other organism which also found conditions favorable for growth in the peritoneum of the mouse.

It is also possible that when a culture was taken from the heart of a mouse, there might have been several organisms present in the blood, one or more of which grew faster than the pneumococci and thus masked their presence.

A fourth factor which was not known to the author until near the end of the survey was that there are different strains of white mice some of which are more susceptible to the pneumococci than others. The mice used in this survey were either raised by the author or were ordered direct from a reputable research supply house. No mention was made when ordering the mice as to the use to which they were to be put.

It is felt by the author that all of these factors should be considered when the results of such a survey are studied. Until one can control more of these variables, the results of such a survey will serve only as an indication of conditions as they probably exist, rather than as picturing the exact state of affairs.

CONCLUSIONS

(1) In a monthly survey from October 1940 to April 1941, 41 (75.9 per cent) of the 54 individuals examined were carriers of *Diplococcus pneumoniae* at one time or another.

(2) Of a total of 351 persons examined, 140 (39.8 per cent) were carriers of pneumococci.

(3) Two methods of obtaining cultures were used, the gargle and the swab method. Although the per cent of positives which each method gave was very nearly alike, the per cent of persons giving positives with both methods at the same time was very low and the total percentage giving positives regardless of method was higher than by either one alone.

(4) To obtain the best results when trying to determine the incidence of pneumococci carriers, both the gargle and the swab methods should be used simultaneously.

(5) There frequently occurred during this survey cultures which were soluble or partially soluble with sodium desoxycholate but which did not give a positive Neufeld reaction.

(6) Nineteen specific types of Diplococcus pneumoniae were found in this survey.

(7) The types of pneumococci found in the order of their frequency are as follows: 3, 17, 6, 8, 4, 13, 28, 20, 19, 15, 31, 24, 18, 32, 25, 9, 16, 5, and 12.

(8) Types 1 and 2 were never found during this survey.

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