

THE OCCURRENCE OF BACILLUS INFLUENZÆ IN THE NORMAL THROAT.

BY AGNES I. WINCHELL AND ERNEST G. STILLMAN, M.D.

(From the Hospital of The Rockefeller Institute for Medical Research.)

(Received for publication, July 15, 1919.)

The present study is a continuation of the work of Pritchett and Stillman on the occurrence of *Bacillus influenzae* in throats and saliva, with additional observations upon the media used and a study of the distribution of *Bacillus influenzae* among the personnel of two institutions.

Pritchett and Stillman¹ found that of 177 persons who gave no history of having had influenza, 74, or 42 per cent, harbored *B. influenzae* in their throats during November and December of 1918. The total incidence of influenza bacillus carriers among 231 normal and late convalescent individuals was 99, or 43 per cent. From 49 cases of uncomplicated influenza, *B. influenzae* was recovered in 41 instances, or 83 per cent, while from 43 cases complicated by bronchopneumonia this organism was cultivated in 40, or 93 per cent. Of 6 cases of bronchopneumonia, which were probably late cases of influenza, all showed influenza bacilli. The incidence of *B. influenzae* was much lower in 20 cases of lobar pneumonia, as only 11 cases, or 55 per cent, were positive. Pneumococci of Types III and IV, which are the groups usually encountered in normal mouths, were the types of pneumococci which were recovered from the cases of influenza complicated with bronchopneumonia.

Methods.

As in the previous study, the medium used in this investigation for the isolation of *Bacillus influenzae* was Avery's² oleate hemoglobin agar. 5 cc. of a 2 per cent solution of neutral sodium oleate were added to 95 cc. of meat infusion agar which made a final concentration of oleate of 1:1,000. 1 cc. of a suspension of rabbit red blood cells was then added while the agar was still hot. Plates were poured

¹ Pritchett, I. W., and Stillman, E. G., *J. Exp. Med.*, 1919, xxix, 259.

² Avery, O. T., *J. Am. Med. Assn.*, 1918, lxxi, 2050.

containing about 15 cc. each. The medium was made fresh each day as the surface soon became dry.

The optimum hydrogen ion concentration for the isolation of influenza bacillus from the throat seems to be between pH 7.2 and 7.5. In every test of medium which was more alkaline than that having a pH of 7.5, unsatisfactory results were obtained. In one instance cultures were made on agar at pH 7.9 from 12 individuals who previously had been persistent carriers. Though the cultures were repeated, only 2 showed small colonies of *Bacillus influenzae*. But, when repeated a third time on medium with a pH of 7.5, 8 were found

TABLE I.
Comparison of Cultures upon Media of Different Hydrogen Ion Concentrations.

Culture No.	pH 8.0		pH 7.4	
	Series 1.	Series 2.	Series 1.	Series 2.
1	—	—	++	++
2	—	—	++	++
3	—	—	—	—
4	—	—	—	—
5	—	+	++	+
6	—	—	+	++
7	—	—	++	+
8	—	+	++	+
9	—	—	++	++
10	—	—	—	++
Total positive.	0	2	7	8

+ indicates colonies of *B. influenzae* present; ++ many colonies of *B. influenzae* present.

to be carrying influenza bacilli. Of 36 other individuals chosen at random, 14, or 38 per cent, were positive when agar at pH 7.4 was used, but only 4, or 11 per cent, with agar at pH 7.9. In Table I are given the results obtained in an examination of 10 individuals from whom both positive and negative plates had previously been obtained. Cultures were taken twice during the same day upon medium at pH 8.0 and at pH 7.4. No positive plates were found in Series 1 on medium with a pH of 8.0, but in Series 2, 2 plates contained several small colonies. On the other hand, Series 1 with 7.4 pH agar shows 7 positive plates, and Series 2, 8 positive plates.

Negative results are so consistently obtained with medium having a reaction above pH 7.5 that one is led to question the value of all negative results obtained upon medium with an unknown hydrogen ion concentration.

All cultures here reported, unless otherwise stated, were taken from the posterior wall of the pharynx. As shown by Table II cultures from the tonsils proved to be unsatisfactory. In a series of 77 individuals, 3 cultures were made from the throat—1 culture from each tonsil, and 1 from the posterior wall of the pharynx. In 4 individuals positive cultures were obtained on plates from all three sources. In 4 other individuals the cultures from the pharynx and from one or the other tonsil were positive. In 11 individuals only the culture from

TABLE II.
Comparison of Cultures from the Pharynx and the Tonsils.

Source of cultures.	No. positive.
Pharynx and both tonsils.....	4
“ “ one tonsil.....	4
Only tonsil.....	0
“ pharynx.....	11
Total No. of cases positive.....	19
“ “ “ “ negative.....	58
“ “ “ “ examined.....	77

the pharynx showed a growth of influenza bacilli. In no case were the organisms isolated from the tonsil and not from the pharynx. The influenza bacillus can unquestionably be isolated from the tonsils, but as there are so many other colonies on the plates made from the tonsils, the influenza bacilli are either overgrown or the colonies are so small that they are hard to detect. West tubes used in a few instances did not give any more satisfactory results than did the simpler method of pharyngeal cultures. Hence, it seemed unnecessary to subject the large group of individuals studied to the discomfort of nasopharyngeal cultures.

All organisms designated as *Bacillus influenzae* were demonstrated to possess the typical morphological and cultural characteristics of this organism; all cultures were proved to be definitely hemoglo-

binophilic. With the exception of staphylococci and diphtheroids, only Gram-negative organisms were encountered upon the oleate hemoglobin agar plates. In addition to the influenza bacillus, there appeared on these plates various types of Gram-negative cocci of the *Micrococcus catarrhalis* group and Friedländer's bacillus group, and also the unidentified bacillus, described by Pritchett and Stillman, which is differentiated from *Bacillus influenzae* by its hemolytic properties.

RESULTS.

Cultures were taken once a month for a period of 6 months from the throats of the personnel of the Laboratories and the Hospital of

TABLE III.
Incidence of B. influenzae in Convalescent and Normal Individuals.

	Nov. and Dec., 1918.			Jan., 1919.			Feb.			Mar.			Apr.			May.		
	Positive cases.			Positive cases.			Positive cases.			Positive cases.			Positive cases.			Positive cases.		
	No.	No.	Per cent.	No.	No.	Per cent.	No.	No.	Per cent.	No.	No.	Per cent.	No.	No.	Per cent.	No.	No.	Per cent.
Normal individuals.....	177	74	42	127	17	13	122	42	34	109	56	51	96	33	34	86	34	39
Convalescents...	54	25	46	42	1	2	40	16	40	42	19	45	38	15	39	28	13	46
Total.....	231	99	43	169	18	11	162	58	36	151	75	50	134	48	36	114	47	41

The Rockefeller Institute in accordance with the method outlined above. It has been possible to follow but 84 of the original 231 individuals used in the earlier study. Of these, 15 have never yielded a positive result. 9 of 35 individuals positive upon the original examination have never since shown the presence of *Bacillus influenzae* in the throat. Of the total 84, 6 have been positive for 5 months and 13 for 4 months.

During the months from December, 1918, to June, 1919, as shown in Table III, the percentage of carriers in a group of approximately 150 individuals has been 43, 11, 36, 50, 36, and 41, respectively. In the previous study the individuals who gave a history of having had

influenza during the time of the epidemic were classed as convalescents. These same individuals have been followed during the complete period. 46 per cent were positive in November and December, 1918, 2 per cent in January, 1919, 40 per cent in February, 45 per cent in March, 39 per cent in April, and 46 per cent in May.

As previously mentioned, any culture medium which varies from the optimum hydrogen ion concentration is unsuitable for use in the isolation of *Bacillus influenzae* from the throat. The reaction of the medium used in November and December had a pH of 7.2, while that for March, April, and May had a pH of 7.4 and 7.5. The reaction of the medium employed during January and February was not tested, and it is apparent, therefore, that no importance can be attached to the low incidence during January.

TABLE IV.
Incidence of B. influenzae in Cases of Acute Respiratory Infections.

Date.	No.	Positive.	Per cent positive.
<i>1919</i>			
Feb.....	9	5	55
Mar.....	16	13	81
Apr.....	12	8	67
Total.....	37	26	70

As is seen from Table IV, *Bacillus influenzae* was recovered from 70 per cent of the persons suffering from acute respiratory diseases who were admitted to the Hospital of The Rockefeller Institute during the months of February to April. Thus it is seen that during the same months a larger percentage of positive cultures was obtained from patients suffering from acute respiratory diseases than from normal individuals.

During the latter part of January, an epidemic of influenza occurred in a girls' school. While the pandemic of influenza raged in the country surrounding this institution during the fall of 1918, a strict quarantine was maintained and only a few questionable cases developed. While the institution was still under strict quarantine, a teacher who had spent Sunday at home developed influenza. The

first cases developed in her classes, and within 10 days over half the students were ill. The epidemic then subsided as suddenly as it arose. On February 16 throat cultures were taken from 52 girls who were still in the infirmary. Of these, 20, or 38 per cent, showed *Bacillus influenzae*. In one cottage which had been under quarantine on account of trachoma since October, 1918, no cases of influenza developed. Of the 20 girls in this cottage, 5, or 25 per cent, showed *Bacillus influenzae*.

In a boys' orphan asylum where a lax quarantine had been maintained and which gave no history of an epidemic of influenza, 190 throat cultures were taken from boys whose ages varied from 10 to 12 years. *Bacillus influenzae* was recovered from 74, or 39 per cent.

DISCUSSION.

Whatever may be the etiologic relationship of *Bacillus influenzae* to epidemic influenza, there is little doubt of the significance of this organism as a secondary invader in this type of respiratory infection. Facts, therefore, concerning the distribution, occurrence, and persistence of *Bacillus influenzae* in the secretions of the respiratory tract are of importance in epidemiological investigation and in prophylaxis. In a study of the occurrence of *Bacillus influenzae* in throats and saliva during the epidemic of influenza last fall, Pritchett and Stillman found this organism present in 42 per cent of 177 healthy persons from whom no history of respiratory infection was obtainable. These observers found the same organism in the throats of convalescents from influenza in 46 per cent of individuals studied. In the same epidemic period Lord, Scott, and Nye,³ by cultural methods, demonstrated influenza bacilli to be present in the pharyngeal secretions of 76 per cent of 34 healthy men of the Harvard Student Army Training Corps. Opie and his collaborators,⁴ by cultural and mouse inoculation methods, found *Bacillus influenzae* in the mouths of 35.1 per cent of all healthy men examined at Camp Funston.

³Lord, F. T., Scott, A. C., Jr., and Nye, R. N., *J. Am. Med. Assn.*, 1919, lxxii, 188.

⁴Opie, E. L., Freeman, A. W., Blake, F. G., Small, J. C., and Rivers, T. M., *J. Am. Med. Assn.*, 1919, lxxii, 108.

These figures serve to indicate the wide distribution and prevalence of the organism during the severe epidemic of this acute respiratory disease.

Since the group of individuals studied by Pritchett and Stillman comprised for the most part the personnel of The Rockefeller Institute, it has been possible to make repeated cultural examinations of the throats of 84 of the same persons during the 6 months subsequent to the original observation. From this study certain facts of interest have been acquired concerning the duration of the carrier state, and the relative frequency of this organism in normal throats after the subsidence of the epidemic. The present study indicates that the percentage incidence of those harboring *Bacillus influenzae* in the upper respiratory tract is as great during the postepidemic period as it was during the influenzal epidemic. During December, 1918, to June, 1919, the percentage of carriers in a group of 150 individuals has averaged 41 per cent per month. This percentage incidence of healthy persons found to harbor influenza bacilli in their throats and saliva is approximately the same as that recorded by Pritchett and Stillman during the height of the epidemic. In addition, it is of interest that in a boys' orphan asylum in which no case of influenza had occurred during the epidemic, 39 per cent of throat cultures taken from 190 boys showed the presence of *Bacillus influenzae*. This percentage incidence of positive cultures is the same as that found in the examination of 52 convalescents from influenza in an institution for girls in which over half the personnel had suffered from the disease.

Furthermore, it is evident that *Bacillus influenzae* may persist in the throats of healthy carriers for a considerable period of time. In 6 instances individuals have had positive cultures on repeated examinations during 5 months, and in 13 other instances during a period of 4 months. In chronic non-tuberculous disease of the lungs *Bacillus influenzae* is known to persist for long periods of time in the secretions of the respiratory tract. The present study, however, demonstrates that in the absence of a focus of infection this bacillus may dwell on apparently normal mucous membranes for long intervals. Whether the organisms thus encountered represent a variety less parasitic than strains actually associated with pathologic lesions

is a problem requiring greater knowledge concerning the biologic differentiation of bacilli of the hemophilic group than is now available.

The technical difficulties incident to the isolation of *Bacillus influenzae*, particularly from the mixed flora of the upper respiratory tract, have been materially lessened by the use of oleate hemoglobin agar. The optimum hydrogen ion concentration for this medium is shown to lie between pH 7.2 and 7.5. Any variation beyond this range, particularly on the alkaline side, reduces markedly the suitability of this medium for growth of *Bacillus influenzae*. Oleate hemoglobin agar not only enhances the growth of the influenza bacillus, but by inhibiting the growth of other organisms, such as streptococci and pneumococci, greatly facilitates the isolation of *Bacillus influenzae* from a focus which harbors a wide variety of other bacteria.

CONCLUSIONS.

1. The frequency of occurrence of *Bacillus influenzae* in the throats of normal individuals has been as great in the period subsequent to the epidemic of influenza as it was during the period of the epidemic.
2. *Bacillus influenzae* may persist in the throats of healthy carriers for a considerable period of time.
3. A considerable proportion of individuals not exposed during the epidemic has been found to carry *Bacillus influenzae* in their throats.
4. Oleate hemoglobin agar has been found to be a very satisfactory selective medium for cultivation of *Bacillus influenzae* from the throat. To give optimum results it should have a reaction of from pH 7.2 to 7.5.