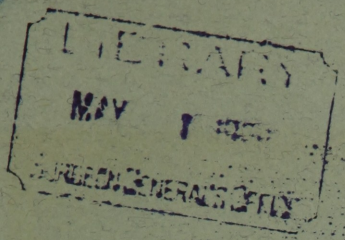

III. THE FATE OF INFLUENZA BACILLI INTRODUCED INTO THE UPPER AIR PASSAGES

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[From The Johns Hopkins Hospital Bulletin, Vol. XXXI, No. 349, March, 1920]

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In previous papers[1] we reported observations on the fate [85]
of certain bacteria after their introduction into various parts of the upper air passages. This work represented a portion of a systematic study of one phase of infection, namely, the period from the arrival of the organism at the mouth or nose until it is eliminated or until disease is produced. *Sarcina lutea, B. coli,* and *Staph. albus* were studied. It was found that these bacteria were rapidly disposed of; *Sarcina* was promptly destroyed by the saliva, and *B. coli* and *Staph. albus* were washed away by mechanical processes without showing any tendency to colonize. The present report deals with a similar study of the hemoglobinophilic bacteria (*B. influenzæ*). No attempt was made to produce disease, and our purpose was simply to study the fate of the organisms and the method of their disposal.

LITERATURE

Several records of inoculations of human beings with *B. influenzæ* are found in the recent literature. These experiments were all made on the hypothesis that the organisms used might be the cause of epidemic influenza and in the attempt to demonstrate this fact by the production of disease. Rosenau[2] introduced strains of influenza bacilli freshly isolated from cases of epidemic influenza into the throats of volunteers without producing any local or general disease. Sellards and Sturm,[3] in the course of a study of hemophilic bacilli isolated from cases of measles, sprayed a saline emulsion of five strains upon the mucous membranes of the eye,

[85] nose and throat of four volunteers. Cultures made at three-day intervals for a period of two weeks were uniformly negative, and no local or general symptoms were produced. Sellards reports no observations made during the first three days after inoculation. Wahl, White and Lyall[4] found that the application to the mucous membrane of the nares and naso-pharynx of a saline emulsion of strains from cases of epidemic influenza failed to produce any abnormal symptoms in five healthy men. These workers were unable to recover *B. influenzæ* from the nose after 48 hours except in one case, but found the organisms present in the naso-pharynx for two weeks or longer. In some cases the bacteria disappeared after a few days to return later. In summary, then, the above experiments, while indicating the general trend of events, give no detailed information about the immediate and exact fate of the influenza bacillus.

METHODS

Strains.—Until the needs of the recent epidemic stimulated the development of media satisfactory for the growth of *B. influenzæ,* little success had been met with in the study of the finer details of its natural history or in the differentiation of strains by biological or other methods. All small Gram-negative hemophilic bacilli isolated from the respiratory passages were placed together in one group. Recent work, however, particularly that of Rivers,[5] suggests that all the organisms previously included under one head are by no means identical, but that they represent various groups differing in essential biological characteristics. It is therefore impossible to be certain now of the exact nature of the original organism of Pfeiffer as well as of the nature and identity of the "influenza bacilli" which were so prominent in the cases of epidemic disease in 1918.

Three strains were employed in the present work. They were all isolated from the throats of healthy men,* and conformed to all the usual criteria of the influenza bacillus group.

Media.—In view of the difficulty of growing influenza bacilli under unfavorable conditions, the utmost care was used in the preparation of the media. Fresh 2 per cent meat infusion

* We are indebted to Dr. Rivers for these strains.

agar (pH 7.3 to 7.5) to which 1 per cent of fresh defibrinated [85]
rabbit's blood was added, or the sodium oleate hemoglobin agar of Avery were used in all the work. The medium was always fresh and moist, and each lot was tested by inoculation with the various strains. It was considered unsatisfactory unless the colonies were large and reached their maximum growth in from 24 to 36 hours.

Inoculations.—As in the previous work, healthy individuals presenting no unusual abnormality of the upper air passages were used. The whole 24-hour growth from an agar slant was collected on a loop and deposited on the desired site—the tongue, nasal septum, naso-pharynx or tonsil crypt. Cultures were made at various intervals by scraping the site of inoculation with a platinum loop or (in the case of the naso-pharynx) by means of the usual cotton swab. The material collected was spread with a glass rod over two plates. It was regarded as essential that the spread of colonies be discreet; plates covered by a confluent mass of growth are useless in work of this sort. The cultures were studied after from 24 to 48 hours, and in every case the diagnosis of *B. influenzæ* was established by isolation of the organism in pure culture. Great care was taken in estimating the quantitative relations.

The site of inoculation was watched for signs of reaction. In no case was there any change in the appearance of the mucosa, or any constitutional reaction.

EXPERIMENTS

Exp. I.—Fate of influenza bacilli swabbed on the tongue. The bacteria were placed on the tongue, and cultures were made from the tongue and from the pharynx at various intervals. The results are summarized in Table I.

Summary.—Influenza bacilli swabbed on the tongue in large amounts were promptly spread over the mouth cavity. The organisms disappeared both from the tongue and pharynx very [87]
rapidly. After two hours the number recovered was much reduced, and after 24 hours only a very few organisms were obtained in two out of four cases. Control cultures before inoculation yielded a very few colonies from the pharynx in two cases. The general result of this experiment is that in 24 to 48 hours the inoculated organisms have disappeared or at least been reduced to the number " normally " present in the pharynx. There was no local or general reaction following inoculation.

TABLE I.—FATE OF B. INFLUENZÆ SWABBED ON THE TONGUE

Name	Date	Procedure	Number of colonies of B. influenzæ per plate recovered from tongue and pharynx												Control culture before inoculation	
			Tongue						Pharynx							
			After 10 min.	After 2 hrs.	After 1 day	After 2 days	After 3 days	After 4 days	After 10 min.	After 2 hrs.	After 1 day	After 2 days	After 3 days	After 4 days.	Tongue	Pharynx
M.	Nov. 10.	One slant strain 55 B. swabbed on anterior half of tongue.	∞*	A good many colonies B. infl.	A very few colonies B. infl.	No B. infl.	....	....	A good many colonies B. infl.	No B. infl.	No B. infl.	No B. infl.	....	....	No B. infl.	A few cols. B. infl.
J.	Nov. 16.	One slant strain 55 B. swabbed on anterior half of tongue.	∞	A very few cols. B. infl.	No B. infl. found.	....	....	No B. infl.	∞	150 cols. B. infl.	20 cols. B. infl.	.....	....	No B. infl.	No B. infl.	A few cols. B. infl.
C.	Nov. 17.	One slant strain 63 swabbed on anterior half of tongue.	∞	150 cols. B. infl.	No B. infl.	....	....	....	A few cols. B. infl.	A few cols. B. infl.	No B. infl.	.....	....	....	No B. infl.	No B. infl.
B.	Nov. 17.	One slant strain 33 swabbed on anterior half of tongue.	∞	A few cols. B. infl.	No B. infl.	....	....	....	Many cols. B. infl.	A few cols. B. infl.	No B. infl.	.....	....	....	No B. infl.	No B. infl.

* ∞ = innumerable.

TABLE II.—THE FATE OF INFLUENZA BACILLI INTRODUCED INTO THE NOSE

Name	Date	Procedure	Number of colonies of B. influenzæ per plate recovered from nose and pharynx										Control culture before inoculation	
			Nose				Pharynx							
			After 10 min.	After 2 hrs.	After 1 day	After 2 days	After 10 min.	After 2 hrs.	After 1 day	After 2 days	After 4 days	After 8 days	Nose	Pharynx
C.	Nov. 11.	One slant strain 56 B. swabbed on left nasal septum.	∞	∞	1000 cols. B. infl.	No B. infl.	.....	A few cols. B. infl.	No B. infl.	No B. infl.	.....	.....	No B. infl.	A few cols. B. infl.
S.	Nov. 12.	One slant strain 55 B. swabbed on left nasal septum.	∞	200 cols. B. infl.	No B. infl.	No B. infl.	.....	No B. infl.	A few cols. B. infl.	About 50 cols. B. infl.*	.....	No B. infl.	No B. infl.	No B. infl.
St.	Nov. 13.	One slant strain 55 B. swabbed on left nasal septum.	∞	200 cols. B. infl.	20 cols. B. infl.	No B. infl.	.....	No B. infl.	No B. infl.	No B. infl.	.....	.....	No B. infl.	No B. infl.
Ch.	Nov. 18.	One slant strain 63 swabbed on right nasal septum.	∞	50 cols. B. infl	No B. infl.	.....	.....	About 20 cols. B. infl.	No B. infl.	.....	.....	.....	No B. infl.	A few cols. B. infl.
B.	Nov. 19.	One slant strain 33 swabbed on left nasal septum.	∞	Several hundred cols. B. infl.	No B. infl.	.....	.....	No B. infl.	No B. infl.	.....	.....	.....	No B. infl.	No B. infl.

* This strain was not 55 B. (See Table V.)

TABLE III.—FATE OF INFLUENZA BACILLI INTRODUCED INTO TONSIL CRYPTS

Name	Date	Procedure	Number of colonies per plate of B. influenzæ recovered from crypt and pharynx													Control culture before inoculation	
			Crypt							Pharynx							
			After 10 min.	After 2 hrs.	After 1 day	After 2 days	After 4 days	After 6 days	After 8 days	After 10 min.	After 2 hrs.	After 1 day	After 2 days	After 4 days	After 6 days	After 8 days	Crypt	Pharynx
Co.	Nov. 25.	One loop strain 33 placed in a tonsil crypt.	300 cols. B. infl.	10 cols. B. infl.	No B. infl.	....	....	...	....	Many cols. B. infl.	Many cols. B. infl.	No B. infl.	.....	.....	.....	.....	No B. infl.	No B. infl.
H.	Nov. 29.	One loop strain 63 placed in a tonsil crypt.	∞	∞	....	100* cols. B. infl.	200* cols. B. infl.	No B. infl.	No B. infl.	Several hundred cols. B. infl.	Several hundred cols. B. infl.	....	Several* hundred cols. B. infl.	Several* hundred cols. B. infl.	About* 100 cols. B. infl.	About* 100 cols. B. infl.	No B. infl.	A few cols. B. infl.
W.	Dec. 6.	One loop strain 55 B. placed in a tonsil crypt.	About 100 cols. B. infl.	A few cols. B. infl.	No B. infl.	....	....	....	....	Many cols. B. infl.	A few cols. B. infl.	No B. infl.	.....	.....	.....	.....	No B. infl.	No B. infl.

* Not strain 63. (See Table V.)

Exp. II.—Fate of influenza bacilli introduced into the nose. The [87] bacteria were deposited on the nasal septum behind the vestibule and cultures were made at various intervals from the nose and pharynx. The results are summarized in Table II.

Summary.—Large numbers of *B. influenzæ* swabbed on the nasal septum disappeared rapidly from the site of inoculation. None could be recovered after 48 hours. Simultaneous cultures from the pharynx in three of five cases yielded a few colonies of *B. influenzæ* in from two hours to 24 hours after inoculation. In one case (S.) 50 colonies were recovered from the pharynx in the 48-hour culture, but this organism was found to be a different strain from that introduced (see Table V).

Exp. III.—Fate of influenza bacilli introduced into tonsil crypts. The bacteria were introduced into a tonsil crypt with a platinum loop and cultures were made at various intervals from the crypt and from the pharynx. The results are summarized in Table III.

Summary.—Influenza bacilli introduced into tonsil crypts could not be recovered from the site of inoculation after 24 hours in two of three cases. In the third case influenza bacilli were recovered after two and also after four days, but they were found to be different strains from those introduced (see Table V). In this case influenza bacilli were also recovered from the pharynx several days after inoculation, but the strain was not only different from that introduced, but it was also different from that obtained from the crypt after two and after four days (see Table V).

Exp. IV.—Fate of influenza bacilli introduced into the naso-pharynx. Influenza bacilli were swabbed on the naso-pharynx and cultures were made at various intervals. The results are summarized in Table IV.

Summary.—Influenza bacilli swabbed in large amounts on the naso-pharynx disappeared very rapidly. After two hours very few organisms could be recovered from the site of inoculation. In one case, influenza bacilli recovered after one and after four days were different strains from those introduced (see Table V).

DISCUSSION

The general result of these experiments seems to indicate two facts: First, that several strains belonging to the group of so-called influenza bacilli swabbed in large amounts on the normal mucous membranes of the upper air passages failed to colonize and disappeared in about a day; and secondly, that in no case did they produce any demonstrable local lesion or general reaction.

TABLE IV.—FATE OF INFLUENZA BACILLI INTRODUCED INTO THE NASO-PHARYNX

Name	Date	Procedure	Number of colonies per plate recovered from naso-pharynx					Control culture before inoculation
			After 10 min.	After 2 hrs.	After 1 day	After 2 days	After 3 days	
W.	Dec. 1.	One slant of strain 33 swabbed on naso-pharynx.	∞	200 cols. B. infl.	No B. infl.	No B. infl.	.....	No B. infl.
R.	Dec. 1.	One slant of strain 33 swabbed on naso-pharynx.	∞	10 cols. B. infl.	A very few* cols. B. infl.	.....	4 cols. B. infl.*	No B. infl.

* Not the strain which was introduced. (See Table V.)

TABLE V.—COMPARISON OF CHARACTERISTICS OF STRAINS OF INFLUENZA BACILLI INTRODUCED AND RECOVERED IN VARIOUS CASES

Name	Characteristics of	Morphology	Staining	Hemoglobinophilia	Hemolysis	Indol formation	Nitrite formation	Agglutination with stock serum
Sp.	Strain introduced (55 B).	Small regular bacilli.	Gram-neg.	Hemoglobinophilic	Non-hemolytic.	*Marked.*	Marked.	+
	Strain recovered from throat 48 hrs. after inoculation.	Small regular bacilli.	Gram-neg.	Hemoglobinophilic	Non-hemolytic.	*None.*	Marked.	0
Ha.	Strain introduced (63).	Small regular bacilli.	Gram-neg.	Hemoglobinophilic	*Non-hemolytic.*	*None.*	Marked.	0
	Strain recovered from throat 4 days after inoculation.	Small regular bacilli.	Gram-neg.	Hemoglobinophilic	Non-hemolytic.	*Marked.*	Marked.	0
	Strain recovered from crypt 4 days after inoculation.	Large thick bacilli.	Gram-neg.	Hemoglobinophilic	*Hemolytic.*	*Marked.*	Marked.	0
R.	Strain introduced (33).	Small regular bacilli.	Gram-neg.	Hemoglobinophilic	Non-hemolytic.	*Marked.*	Marked.	0
	Strain recovered from throat 24 hrs. after inoculation.	Very large numerous threads.	Gram-neg.	Hemoglobinophilic	Non-hemolytic.	*None.*	Marked.	0
	Strain recovered from throat 3 days after inoculation.	.....	Gram-neg.	Hemoglobinophilic	Non-hemolytic.	*None.*	Marked.	0

TABLE VI.—EFFECT OF SALIVA ON INFLUENZA BACILLI

Date	pH. of saliva	Strain of B. infl.	Time of culture after inoculation	0.5 c. c. broth and growth from one slant B. infl.	0.5 c. c. saliva and growth from one slant B. infl.
Dec. 6.	7.0	55 B.	Immediately.	Innumerable cols. B. influenzæ.	About 50 cols. "mouth bacteria" per plate + innumerable cols. B. influenzæ.
			After 2 hrs.	Innumerable cols. B. influenzæ.	About 50 cols. "mouth bacteria" per plate + innumerable cols. B. influenzæ.
			After 24 hrs.	Innumerable cols. B. influenzæ. (About same number as in previous culture.)	About 300 cols. "mouth bacteria." *No* B. influenzæ.
Dec. 6.	7.0	33	Immediately.	.....	About 100 cols. "mouth bacteria" per plate + innumerable cols B. influenzæ.
			After 2 hrs.	.....	About 75 cols. "mouth bacteria" per plate + innumerable cols. B. influenzæ.
			After 24 hrs.	.....	About 500 cols. "mouth bacteria" per plate. *No* B. influenzæ.
Dec. 3.	7.3	63	Immediately.	.....	About 300 cols. "mouth bacteria" per plate + innumerable B. influenzæ.
			After 2 hrs.	.....	About 300 cols. "mouth bacteria" per plate + innumerable B. influenzæ.
			After 24 hrs.	.....	About 300 cols. "mouth bacteria" per plate. *No* B. influenzæ.
Dec. 3.	7.3	33	Immediately.	.....	About 50 cols. "mouth bacteria" per plate + innumerable B. influenzæ.
			After 2 hrs.	.....	About 75 cols. "mouth bacteria" per plate + innumerable B. influenzæ.
			After 24 hrs.	.....	About 500 cols. "mouth bacteria" per plate. *No* B. influenzæ.
Dec. 4.	6.4	55 B.	Immediately.	.....	About 100 cols. "mouth bacteria" per plate + innumerable B. influenzæ.
			After 2 hrs.	.....	About 75 cols. "mouth bacteria" per plate + innumerable B. influenzæ.
			After 24 hrs.	.....	About 100 cols. "mouth bacteria" per plate. *No* B. influenzæ.

[87] In five instances influenza bacilli were recovered at periods later than 24 hours after inoculation. It seemed of importance to determine whether these strains were identical with those introduced or whether the strain introduced had disappeared and another had supplanted it. Biological differential methods worked out by Dr. Rivers were applied by him to the study of these cultures. In every case it was possible to show that the strain introduced had been replaced by organisms possessing different characteristics (see Table V). It seems desirable to bring these findings into relation with the known facts about the presence of influenza bacilli in the throats of healthy people. Winchell and Stillman [6] review the recent literature on this subject and report careful studies of their own on a series of individuals over a period of eight months. It seems quite certain that hemophilic bacilli may be found in from 40 to 80 per cent of various groups of healthy people. The exact seat of bacterial multiplication in the pharynx and the length of time over which any given strain persists are however unknown. Our experiments suggest that the free surfaces of the normal mucous membranes present a relatively unfavorable environment for these bacilli to colonize upon. It may be that their source in certain cases is a focus, such as a chronic sinusitis, bronchitis, or bronchiectasis, or an acute infection of the respiratory tract either in the same or in another individual from which they are discharged into the open pharyngeal cavity. It should be emphasized that all studies on the persistence of influenza bacilli in the throat are of uncertain significance in the absence of proof that the strains recovered from the same individual at various times are identical. The final solution of this question must await the development of complete and accurate methods of differentiating the various strains of hemophilic bacilli.

Inasmuch as the organisms employed in our experiments had been grown on artificial media for several generations the objection may be raised that their failure to colonize was due to alteration in their virulence by growth outside the body. This objection cannot be answered and the experiments are presented with this in mind.

As in the case of the organisms previously studied an attempt was made to analyze the various factors which might be responsible for the disposal of *B. influenzæ.*

1. *The Antagonistic Action of Other Bacteria in the Mouth and Throat.*—No direct experimental method was available for determining to what extent the growth and multiplication of *B. influenzæ* in the throat is prevented by bacteria already present. It is known that *B. influenzæ* grows well on artificial media in symbiosis with many other bacteria under proper quantitative relations. It may, however, be rapidly overgrown by an excess of other bacteria which in smaller numbers would favor its growth. In the case of nasal inoculations other bacteria can hardly play an important part since the nasal mucosa is practically free of organisms.

2. *The Effect of the Mouth Secretions.*—An attempt was made to reproduce mouth conditions by testing the effect of saliva on influenza bacilli in the test tube. Fresh saliva was centrifuged at high speed for a few minutes to remove gross particles. Small amounts of the resulting clear fluid were placed in test tubes and heavily inoculated with *B. influenzæ.* The entire growth from an agar slant was suspended in 0.5 c. c. of saliva, and control tubes of plain broth were similarly inoculated. Cultures were made at various intervals on oleate hemoglobin agar plates. Three salivas covering the normal range of reaction (pH 6.4 to pH 7.3), and three strains of [89] influenza bacilli were employed. The results are summarized in Table VI.

This experiment shows the following points. Influenza bacilli suspended in plain meat infusion broth were viable at the end of 24 hours. From saliva inoculated with the same amount of culture living influenza bacilli were recovered after two hours, but not after 24 hours. The proportion of mouth bacteria in the saliva to the inoculated influenza bacilli was about 1 to 10,000 at the beginning of the experiment. At the end of 24 hours there was only a slight increase in the number of mouth bacteria, whereas no influenza bacilli grew. The experiment seems therefore to indicate that while saliva exer-

[89] cises no immediate destructive effect on *B. influenzæ,* it is an unfavorable medium for the growth of these bacteria and that they do not remain viable in this medium for as long as 24 hours. This result was constant with salivas of different pH.

The probable explanation, therefore, of the rapid disappearance of *B. influenzæ* when introduced into the normal upper air passages involves both the action of a medium unfavorable for the growth of these organisms, together with the mechanical flushing processes constantly at work in these regions.

The question naturally arises in this connection as to what makes possible the tremendous growth of influenza bacilli in disease conditions, such as acute sinusitis, pharyngitis, laryngitis, pneumonia, measles, influenza, etc. A possible explanation which still lacks final proof is that these acute processes may alter the environment in such a way that the organisms take hold and grow rapidly at the seat of disease.

SUMMARY

1. Three strains of influenza bacilli introduced in large amounts into the normal upper air passages disappeared very rapidly—within from one to two days. In no case was a carrier state produced.

2. In no case did any local or general pathological process result from such inoculation.

3. In five instances influenza bacilli isolated later than 24 hours after inoculation were shown to be different strains from those introduced.

4. Influenza bacilli were no longer viable after being suspended in saliva for 24 hours at 37°C.

5. The rapid disappearance of influenza bacilli from the upper air passages is probably due to the combination of an unfavorable environment with the mechanical flushing processes at work in these regions.

6. The question of the persistence of influenza bacilli in normal throats cannot be finally settled until we possess accurate methods for differentiating various strains of hemophilic bacteria.

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