INTRODUCTION.

Since the first paper in the series by Olitsky and Gates (1) in which the presence of minute filter-passing bodies (*Bacterium pneumosintes*) in cultures from affected rabbits' lungs and from filtered nasopharyngeal washings of influenza patients was reported, a number of investigators in widely separated laboratories have described the isolation, under similar conditions, of anaerobic filter-passing organisms, identified morphologically and sometimes serologically as *Bact. pneumosintes*. Such organisms were "obtained consistently" by Loewe and Zeman (2) in New York, from the filtered nasopharyngeal washings of patients with epidemic influenza, and produced a characteristic clinical and pathological picture (3) when injected into experimental animals. Then Gordon (4) in London reported evidence of the same bacterium in 14 of 20 influenza cases, and filtrates of the bronchial secretion in 3 fatal cases yielded 2 cultures. In 1922 also, Lister (5), in South Africa, obtained 5 cultures of an identical anaerobe in 11 instances in which influenzal washings were cultured within 24 hours of the acute onset, and reported 4 febrile reactions, 1 fever and drop in the leucocyte count, and 1 case of typical influenza among 12 volunteers sprayed with unheated cultures of the filter-passing organism. No reactions occurred among 6 volunteers sprayed with heated cultures. In 1923 Nakajima (6) in Tokio cultivated 2 strains from pharyngeal washings and 1 from the lung tissues of a fatal case of influenza. Seitz (7) in Zurich observed masses of very tiny bodies in the respiratory exudates of influenza patients and grew them for a time in mixed sputum cultures. He regards these bodies as coccoid
rather than bacillloid. Detweiler and Hodge (8) in Toronto grew 3 strains morphologically similar to \textit{Bact. pneumosintes} from filtered influenza material, 2 from lung filtrates of injected animals, 1 from filtered nasopharyngeal washings. Subcultures failed to grow, so identification was not completed. Finally Thomson (9) isolated a minute organism from a case of Engadine fever—a type of influenza endemic in Switzerland—and obtained 2 other strains from influenza patients in England.\footnote{While this paper was in press M. W. Hall (\textit{J. Exp. Med.}, 1926, xlv, 539) reported the experimental production of characteristic lung lesions in rabbits and guinea pigs with nasopharyngeal washings from a patient with typical epidemic influenza. From one of his affected animals a culture of \textit{Bact. pneumosintes} was obtained. The presence of this organism in the lungs of experimentally infected animals predisposed them to the pulmonary localization of other bacteria and the production of definite secondary pneumatic lesions.} In all of these studies control experiments with non-influenzal material have been uniformly negative.

Thus the presence of an anaerobic, Gram-negative, filter-passing bacterium, identified as \textit{Bact. pneumosintes}, in the human respiratory tract only in the early hours of an influenzal infection, has been established in many parts of the world. This in itself is but a beginning, however, in determining the relationship of \textit{Bact. pneumosintes} to the clinical disease. A large accumulation of observational and experimental evidence must be sought wherever available, and pieced together as opportunities permit.

\textit{The Outbreak of 1926.}

When we undertook an investigation of the presence of \textit{Bact. pneumosintes} in clinical influenza in New York City last March (1926), the brief local outbreak proved to be already on the wane. Consequently we had an opportunity to see only a few cases. It was reported that clinically the infections varied considerably in mode of onset, relative prominence of various signs and symptoms, and the blood picture. Yet they would be grouped together with the common designation "influenza" as typified by sudden onset, sometimes with chill, a sharp fever and marked prostration, headache and other pains, absence of profuse coryza, and prolonged depression during convalescence. Sometimes family infections, and more often isolated...
cases occurred. Compared with the influenza epidemic and its reper-
cussions of 1918 to 1922 this outbreak was characterized in general
by the relative mildness of the primary infection and the rarity of
secondary complications. Often other signs of typical epidemic
influenza, such as flush, photophobia, conjunctivitis, diffuse pharyngi-
tis, and the characteristic leucopenia, were noted, but in the absence
of acknowledged proof of the etiologic agent in clinical influenza it
would be unwise to draw a close parallel between the infections of this
transient outbreak and influenza of the epidemic type. A search for
*Bact. pneumosintes* in these sporadic cases thus presented a new field
for study and a new problem in its relation to the clinical disease.

In the 10 days that elapsed between our first contact and the failure
of available material we obtained nasal and postnasal washings from
9 patients with clinical influenza and from 1 person who developed
only a common cold. In several instances the patients had com-
plained of a headache and feeling of depression for a day or two before
the acute attack, so that the actual time of invasion is difficult to
determine, but the washings were obtained in each instance within
24 hours of the acute onset, marked by fever and prostration in bed.
We decided at the outset to follow the established routine in detail
and so handled our material as follows:

The patient's nose and throat were washed out with 40 to 50 cc. of sterile,
dextrose Ringer's solution. The washings were shaken with beads and divided
into two portions. One sample was filtered through a new Berkefeld V candle
and used to inoculate Smith-Noguchi medium and *coli* broth (10) under a vaseline
seal, and was spread on rabbit blood agar plates for aerobic and anaerobic incu-
bation. The unfiltered nasopharyngeal washings were injected intratracheally
by the method of tracheotomy into stock rabbits under light ether anesthesia.
The febrile and leucocytic reactions of these rabbits were carefully followed and
at autopsy on the 1st or 2nd day, portions of the lung tissue of these animals were
ground, and this material, filtered or unfiltered, was inoculated into Smith-
Noguchi tubes and on blood agar plates. Fragments were also placed in 50 per
cent glycerol, and the rabbits were examined carefully for evidence of concurrent
disease.

*Rabbit Passages.*

Unfiltered washings from 7 of the 9 influenza patients were injected
intratracheally into 13 rabbits, usually in amounts of 3 cc. 9 of these
rabbits, representing 6 patients, showed significant reactions, such as fever, a drop in the leucocyte, and especially the monocyte, count, and typical gross and petechial hemorrhages in large, edematous lungs. 6 rabbits injected with whole or filtered lung tissue from 4 of these animals showed similar but less striking effects in the 2nd passage. A 2nd passage apparently failed in 4 other transfers from 2 of the other 5 rabbits.

This series of rabbit injections was carried out under a serious handicap. Our stock of rabbits was low, and the immediate demands of the situation required the use of untested animals. Although only apparently healthy rabbits were chosen for injection, the whole lung tissues of most of them subsequently showed infection with *B. lepi-septicus* or *B. bronchisepticus* and cultures of them had to be discarded. In the presence of these concurrent infections a strict interpretation of the reactions of most of these rabbits is not justified. The series was therefore discontinued and our attention turned to the direct cultivation of filter-passing, anaerobic organisms from the nasopharyngeal washings, and from filtered material from the rabbits' lungs.

*Cultivation Experiments.*

Having in mind that often very sparse growths of filter-passing anaerobes are obtained in early generations, and might easily be missed in the Smith-Noguchi medium, we made at least 2 successive transfers of every primary tube that showed no growth when subplanted on aerobic blood agar plates. The control tubes set up to test the sterility of our media were likewise transferred, to avoid the possibility of false evidence from an extraneous source. Material from each generation was also examined microscopically in stained smears.

This tedious procedure proved to be justified in 6 series of cultures in which minute anaerobic organisms were obtained. 2 of these have been definitely identified as strains of *Bact. pneumosintes*. In the first 2 generations of these *pneumosintes* cultures the growth was so sparse as to escape microscopic detection and no visible colonies developed in subplants on anaerobic blood agar plates. But in the 3rd, 4th, and subsequent generations the typical clouding of the Smith-
Noguchi medium, the microscopic observation of minute, Gram-negative bodies such as have been fully described (10), and the growth on anaerobic blood agar plates of microscopic, discrete, round, convex colonies with an entire edge and a colorless translucency indicated the growth of \textit{Bact. pneumosintes} morphologically identical with the 1918 to 1922 strains.

One strain was obtained from the whole lung tissue of a rabbit injected with unfiltered nasopharyngeal washings, and consequently had not been filtered. Fortunately this rabbit was free from previous lung infection and, as in numerous cases reported by Olitsky and Gates (11), the contaminating bacteria (in this case \textit{S. albus} and diphtheroids) in the unfiltered nasopharyngeal washings were suppressed during the rabbit passage.

The 2nd strain, also obtained through rabbit passages, was derived from the filtered lung tissue of a 2nd passage rabbit, intratracheally injected with whole lung tissue that had stood in 50 per cent glycerol for 31 days. This 1st passage lung tissue had been contaminated with a large Gram-negative bacillus which did not survive glycerolation, so that the lungs of the 2nd rabbit yielded no aerobic growth. The 2nd passage rabbit showed no fever, only a slight leucopenia (a drop in monocytes from 2790 to 2040 cells), and no gross lesions except one small surface hemorrhage in the lungs.

No primary cultures of \textit{Bact. pneumosintes} were obtained in \textit{coli} broth or on anaerobic blood agar plates. These media are only suitable for special purposes with well established strains. This fact emphasizes the importance of the Smith-Noguchi medium for primary cultures, and even in this medium the initial cultivation of \textit{Bact. pneumosintes} is difficult and uncertain. A lesson may be drawn from the detection of this fastidious organism only in the 3rd generation of culture and even then only after 1 or 2 preliminary rabbit passages. It was our earlier experience that the cultivation of \textit{Bact. pneumosintes} was more frequently successful from the lung tissues of affected rabbits than directly from the filtered nasopharyngeal washings of influenza patients.

2 other strains of anaerobic, filter-passing organisms morphologically similar to \textit{Bact. pneumosintes} were obtained in Smith-Noguchi medium directly from the filtered nasopharyngeal washings of other
influenza patients. The 1st generations grew so sparsely as to escape
detection and the bacteria were first discovered as submicroscopic
colonies in subplants on anaerobic blood agar plates. Although these
2 strains have grown well in successive generations on solid media in
the anaerobic jar, they have both died out in the Smith-Noguchi tubes
and repeated attempts to reestablish growth in fluid media have so
far failed. The morphological similarity to \textit{Bact. pneumosintes}, the
very minute, discrete colonies on blood agar plates, and the failure to
develop in successive transplants on fluid media are characteristic of
the Group II organism briefly described by Olitsky and Gates in 1922
(12). These strains have not yet been grown in sufficient quantity
for serological examination.

In addition to these 4 morphologically similar organisms, which
may all belong to a common group, 2 other anaerobic filter passers
were isolated directly from washings of influenza patients. As in the
earlier studies, the identification of these 2 other organisms depended
on the use of anaerobic blood agar plates on which they grow readily
in visible colony form. The primary cultures were obtained in Smith-
Noguchi medium; in one instance also in \textit{coli} broth. One strain is
apparently a variant of Group I, the other is similar to the organisms
described as Group III (12).

\textit{Serological Reactions.}

At the beginning of this investigation 6 rabbits were set aside for
immunization with old strains of \textit{Bact. pneumosintes} (C 17 and C 34)
from 1919 and 1922. The organisms were grown in \textit{coli} broth, washed,
standardized, and injected subcutaneously in large doses at weekly
intervals until 7 or 8 injections had been given. The rabbits then
yielded serum with a complete agglutination titer of 1:160 to 1:320
against the old strains. These titers are the highest that have yet
been obtained with these organisms and may indicate an increase both
in agglutinogenic properties and in response to serum antibodies on
prolonged saprophytic cultivation.

The 2 new strains of \textit{Bact. pneumosintes} show a strictly specific ag-
glutination in low dilutions, 1:2 to 1:20, of this anti-\textit{pneumosintes}
serum.
At intervals of 15 to 30 days after the acute onset, serum samples were obtained from 8 of the 9 influenza patients from whom washings had been taken, and from 9 other convalescents from clinical influenza. As controls 10 samples were taken from normal persons who said that within a year they had not had any acute respiratory infection diagnosed as influenza. These sera were tested for specific agglutinins, by the method previously described (13), against 2 old strains (Nos. 17 and 34), and against the 2 new strains of Bact. pneumosintes (Nos. 49 and 50), so far as the very limited amounts of available material permitted.

A summary of these agglutination tests (Table I) shows that the serum of only 1 patient with a clinical diagnosis of influenza failed to

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* Group III organism recovered.
* Bact. pneumosintes recovered.
* Group I organism recovered.
* Group II organism recovered.
agglutinate 1 or both old strains of *Bact. pneumosintes*, and when
tested, most of them (12 of 15) agglutinated 1 or both of the 1926
strains also. Considered together with the agglutination of the
new strains by specific anti-*pneumosintes* rabbit serum, this evidence
points to an immunological relationship as well as a morphological
identity between the 1919 to 1922 and the 1926 strains. Heretofore
agglutination of *Bact. pneumosintes* even after a long saprophytic
existence in the laboratory has not been found in the serum of sup-
posedly normal persons. In these tests unquestioned agglutination
of old and new strains sometimes occurred. Several explanations
of this phenomenon are possible, but we shall not attempt to develop
any of them at this time, on the basis of the evidence available at
present.

SUMMARY.

The presence of *Bacterium pneumosintes* has been demonstrated in
nasopharyngeal washings from 2 patients in a sporadic outbreak of
clinical influenza in New York City in March, 1926. 2 strains of
bacteria morphologically similar to *Bact. pneumosintes*, but differing
in certain cultural characters, and 2 other anaerobic filter-passing
organisms were also isolated from the 9 patients examined.

The blood serum of 16 among 17 persons convalescent from clinical
influenza, and of 6 among 10 supposedly normal persons, agglutinated
1 or more strains of *Bact. pneumosintes*.

BIBLIOGRAPHY.

   135.