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SOME EXPERIMENTS ON THE TRANSMISSION OF INFLUENZA *

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The prevalent vague and contradictory conceptions of influenza, its etiologic agent and its mode of transmission are responsible for the absence of uniform and systematic measures for its control and the comparative helplessness of the medical profession in the face of its ravages. Animal experimentation does not lend itself to the use of material or organisms isolated from influenza patients, making the use of human subjects for experiment the best hope for a rational basis for the control of the disease.

While Pfeiffer's bacillus is frequently found in the mucous membrane of the respiratory tract and in the lungs of influenza patients, its relation to the etiology of this disease has not been established, especially in view of the fact that other organisms, such as the pneumococcus, diplococcus, pleomorphic streptococcus, micrococcus catarrhalis, etc., have been reported frequently not only in association with, but also in the absence of, the influenza bacillus. On the other hand, the recent communications of Nicolle and others,[1] brief and inconclusive as their experiments are, lends color at least to the view that the exciting cause may be a filtrable virus, and that the influenza bacillus bears but a symbiotic relationship to it, analogous to B. aertrycke and the virus of hog cholera.

The present investigation was made for the purpose of ascertaining some possible factors in the mode of transmission of influenza through the use of human subjects for experiments. Two objectives were sought: first, to determine the infectious nature of bacteria free-filtrates as reported by Nicolle; second, to test the pathogenicity of

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* The observations recorded in this paper are a portion of a report submitted by a board appointed by Colonel C. F. Craig, commanding officer, Yale Army Laboratory School, New Haven, Conn., to investigate the etiology of influenza. Authorization for the human experimentation that was necessary was granted by the Surgeon-General who authorized the board to call for volunteers for the study of influenza virus. Six officers of the Sanitary Corps of the army responded and the nature of the experiment was explained to each of these officers, together with the risk that they were taking in submitting themselves for inoculation with material supposed to contain the virus, and they all signified their willingness to serve and the result of the experiments is given in this paper.

[1] Compt. rend. Acad. d. sc., 1918, 167, p. 607; Dugarrie de la Riviere, R.: ibid., p. 605.

various typical strains of influenza bacilli for man, and ascertain if their use will result in the production of the disease.

The plan and course of the investigation were:

A. Observation of the Volunteers.—Six apparently normal men (officers assigned to the school), having been fully informed of the possible hazards, volunteered as subjects. They were quartered and strictly isolated in the isolation pavillion of the hospital. Two of the volunteers had not been vaccinated with any influenza vaccine (volunteers 4 and 5), while the others had taken a polyvalent influenza vaccine from 4-6 weeks previously. Volunteer 5 had, however, had pneumococcus vaccine. None had had influenza.

On admission to the hospital all of the men were kept under observation for 4 or more days before being used. Careful physical, clinical and laboratory examinations were made and recorded during this period. Two sergeants were detailed to attend to the wants of these men. Blood was taken for serologic study by Majors Gay and Harris. No antibodies against the influenza bacillus were found.

During the period of observation the bacterial flora of the nares and the nasopharyngeal vault of each volunteer were studied. In swabbing, care was exercised to secure the secretions of the nose and nasopharynx free from incidental contamination from the nostrils or buccal cavity. The material on the swabs was then immediately streaked on plates of (a) whole-blood agar, (b) chocolate agar of Williams,[2] and (c) Avery's oleate hemoglobin agar.[3]

Preparation of Medium.—(a) Blood agar: To every 100 c c neutral beef infusion, 5 c c fresh sterile defibrinated sheep blood was added at 45-50 F., and after thorough mixing poured in the plates.

(b) Chocolate agar: 3 c c fresh defibrinated sheep blood was added to every 100 c c of neutral beef infusion agar containing 5% glycerol. It is important that the blood be added while the glycerol agar is hot; that is, immediately after the latter is taken from the Arnold sterilizer (95 F.). The medium should have a dark chocolate color. If light in color it does not work as well.

(c) Sodium oleate hemoglobin agar (Avery): Add the washed cells of 1 c c fresh sterile sheep blood and 5 c c of a sterile 2% watery solution of neutral sodium oleate to 95 c c of hot (95 C.) 2% infusion agar, neutral to — 0.3% reaction.

In each case the melted medium was cooled to 42 F. before pouring in order to reduce the water of condensation to a minimum. All plates were incubated from 24-48 hours before use. Cultures and plates were held in the incubator (37 C.) and reexamined daily. Colonies were described in the order of their predominance, smears made and examined and subcultures made on chocolate, blood, oleate and infusion-agar plates.

During the period of preliminary observation one of the volunteers (4) developed an acute catarrhal infection of the upper respiratory tract which made it advisable to exclude him from the investigation, even though the symptoms, course and blood picture were not characteristic of influenza. Because of this occurrence the experiments on the other volunteers were postponed four days.

B. Experiments.—*Exper. 1.* The purpose of this experiment was to determine the infectiousness of the filtrates of typical influenza lungs when applied

[2] Park and Williams: Pathogenic Micro-Organism, 1917, p. 103.

[3] Jour. Am. Med. Assn., 1918, 71, p. 2050.

to the nasopharyngeal mucous membrane of man. Two filtrates were prepared; one (152) was obtained from the pneumonic area of a lung from a typical case of influenza pneumonia. The tissue was obtained 4 hours after death. The organisms isolated from the lung were (a) pneumococcus-type 2 (from blood, pleural fluid and lung tissue) . (b) B. mucosus, and (c) M. catarrhalis. No influenza bacilli were identified from the respiratory tract of this case. The other filtrate was taken from the pneumonic area of a typical case (154) of influenza. Influenza bacilli were isolated from this case. In addition a streptococcus viridans, a pneumococcus type 4, and a staphylococcus albus were identified as being present. Filtrates of the sputum were not made because of the lack of suitable material. The material from 154 was obtained about 24 hours after death.

Ten grams of the tissue were finely chopped, ground up with salt solution and sterile sand in a mortar, centrifuged and the supernatant fluid passed through a Berkefeld filter and the clear filtrate collected aseptically. Bacteriologic tests showed it to be sterile.

The filtrate was placed in a DeVilbis atomizer and sprayed heavily in each nostril and the nasopharynx of two of the volunteers (1 and 3); three squeezes of the atomizer bulb were given in each location. The atomizers were tested as to their efficiency and were in good order.

No apparent clinical effect followed the use of the filtrates. No subjective symptoms were noted. The temperature curve was unchanged and the blood picture presented nothing unusual. Daily bacteriologic examinations of the nares and pharyngeal vault both before and after the use of the filtrates showed no noteworthy change either in the way of suppression or stimulation of former types or the appearance of new types of bacteria.

Exper. 2. This consisted in applying various strains of B. influenzae to the nasopharyngeal mucous membrane in order to determine their pathogenicity.

The following typical strains of the influenza bacillus were used: Strain C obtained through the kindness of Dr. George Smith from Dr. William H. Park, isolated in September, 1918, from nasopharynx. Strain L from the same source, Sept. 21, 1918. Strain 69, isolated at this laboratory on Nov. 26, 1918, from the nasopharyngeal secretion at the height of the disease and 24 hours before the death of the patient. This organism was associated with an indifferent streptococcus and M. catarrhalis. This strain was 15 days old when used.

Every precaution was taken that the above strains were pure before use. They were plated, single colonies fished, stained and planted on blood, chocolate, oleate and plain infusion-agar plates. The morphology was typical of Pfeiffer's organism. The profuse 24-hour growth on one plate of chocolate-blood agar was thoroughly emulsified in salt solution and the volume made up to 10 c c, in each case, giving a heavy milky appearing suspension. A loopful of this suspension was spread out on chocolate, oleate and plain infusion-agar plates. In each case typical profuse growths occurred on the first two but no growth on the last.

All (5) of the volunteers were used. The normal bacterial flora were determined by almost daily examinations of the nares and nasopharyngeal vault for a period of from 4-7 days prior to this experiment. As soon as the suspension of the influenza bacilli was made and control plates planted, each was placed in a sterile DeVilbis atomizer and immediately sprayed into each nares and, through the mouth, into the nasopharynx of the proper volunteer by three complete compressions of the atomizer bulb. In each case the

atomizer was tested to see if it was in good order and a heavy spray issued each time. The nares and nasopharynx were swabbed and the material plated just before giving the spray. Strain C was given to volunteers 1 and 6, both inoculated against influenza about 6 weeks ago; but the blood showed no antibodies against Pfeiffer's organism. Strain L was given to volunteer 2. Strain 69 was given to volunteers 3 and 5 (5 being the only one who had not been inoculated with an influenza vaccine).

Culture tests on the atomizer after use showed that B. influenzae was present, viable and in pure culture.

In the next few days no untoward symptoms, abnormal physical or clinical findings were observed. The temperature curve presented its usual normal range. There was no distinct change in the blood picture. In fact, there were no symptoms in the least suggestive of influenza.

On the first day bacteriologic examinations of the nose and throat were made every 4 hours, twice the next day and daily thereafter. There was no material change in the bacterial flora except the addition of the influenza colonies which appeared in all of the plates the first two days after spraying, but then disappeared quite rapidly from the nares, though persisting in the nasopharynx 1 to 3 weeks or even longer, and this even in spite of the use of dichloramin T.

Exper. 3. This was made to determine the pathogenicity of a freshly isolated strain of the influenza bacillus, strain 162, from the unconsolidated portion of the lung of a fatal case of influenza pneumonia in which the influenza bacillus was present in large numbers along with an indifferent streptococcus, staphylococci of both albus and aureus types and a gram-negative bacillus belonging to the Friedländer type. The patient died at 10:30 a. m., and a necropsy was held at 3 p. m. of the same day when the cultures were made. The following morning the chocolate and oleate and blood agar showed many typical influenza colonies. Discrete colonies were fished and spread on oleate and chocolate plates. The following morning the plates showed a pure culture of what were apparently influenza bacilli. A suspension was made from the profuse growth on the chocolate plate and used precisely as in the second experiment. Controls of the contents of the atomizer both before and after use showed that the material used contained viable influenza bacilli in pure culture. Subsequent study proved this strain to be a typical strain of B. influenzae.

The suspension was made and introduced in the volunteers in the same way as in the previous experiment and was given to volunteers 5 and 6 (four days after they had a previous spray of influenza bacilli in experiment 2). This suspension represented the second generation of a strain of influenza bacilli only 43 hours removed from the infected lung.

The temperature curve showed no striking change; number 6 showed a slight rise (0.5 F.) on the afternoon of the same day, and he said he felt as if he had a slight cold in the head, but this disappeared the following day. The rest of the clinical course was uneventful and nothing suggestive of influenza appeared. The bacteriologic findings were identical with those following the second experiment.

The bacterial flora of the nose and throat was studied in detail in order to detect organisms, such as hemolytic streptococci, bacilli of the Friedländer type, pneumococci, M. catarrhalis, etc., that might contribute to complications and particularly for the purpose of determining or excluding a possible symbiotic rôle of any of the bacterial types found.

As stated, the bacterial flora was fairly constant for each individual and remained unchanged after the use of either the filtrate or the suspension of living bacilli, with the exception of the addition of a greater or less number of influenza colonies after the spraying in of these organisms.

The second point is the persistence of influenza bacilli in the nasopharynx as contrasted with their early disappearance from the nose. In the latter (except in 5) they disappeared in less than 72 hours, while in the throat they were present several weeks, and in one case present at the time of writing (1). It may be that staphylococci and diphtheroids, more common in the nose than in the throat, may inhibit the growth of influenza bacilli and be responsible for their early disappearance, whereas the types found in the throat are not so antagonistic. As might be anticipated, more types of bacteria were present in the nasopharynx than in the nares. In the former streptococci of the viridans and indifferent types were almost constantly found, while in the latter they were either absent or when present, found in relatively small numbers. The absence of hemolytic streptococci, pneumococci and bacilli of the Friedländer type is significant in view of the negative findings in this investigation, and their frequent occurrence in influenza patients.

The appearance and persistence of the influenza bacilli after their experimental introduction in the throat is of considerable interest. Not only was the organism present in the nasopharynx of all of the volunteers at the end of 12 days, but it was present in considerable numbers. It is also noteworthy that after 3 or 4 days the organisms became relatively scanty for a day or two and then reappeared in much larger numbers, and even as the predominating organism. This suggests that the mucous membrane of the nasopharynx of man is a favorable habitat for this organism, and that the bacillus not only exists, but multiplies there.

The bacterial flora of volunteer 6 deserves special consideration. In every culture of his nose and throat throughout his observation a small gram-negative bacillus was present which in every respect (except in the form of some of its colonies) would be classified as a strain of the influenza bacillus. It grew more luxuriantly than the other strains used and the moist confluent appearance of its colonies suggested more those of bacilli of the Friedländer group. Yet their consistency was not the same and seemed more like that of B. influ-

enzae. The colonies were not nucleated, and this aided in differentiating them from those which were introduced and which were nucleated colonies. These bacilli consistently failed to grow on plain infusion agar, serum agar, on Loeffler's coagulated serum, or in plain infusion broth. It grew only on mediums containing blood. At times the morphology was confusing because of the pleomorphism it showed. At one time smears from discrete colonies contained only sharply defined, intensely staining bacilli with thread forms, and the majority of the individuals were larger or more sharply defined than was ever observed in the typical strains of B. influenzae. Yet frequently, especially on the chocolate plates, these bacilli had the classical morphology of the influenza bacillus, not only in the short, delicate, slender bacillary and coccal forms, but also in their faintly staining properties. This organism was nonpathogenic for rabbits when given in large doses either intravenously or subcutaneously, which was also true of the four strains of B. influenzae implanted in the nasopharynx of the volunteers. The organism was therefore tentatively classed as an influenza bacillus. Although this man had never had influenza, he had been exposed in the recent epidemic and was also in the sudden transient epidemic of "grippe" at Fort Oglethorpe in the spring of 1918.

Ten days after exper. 2 was begun, none of the volunteers showing any symptoms, but all showing more or less heavy growth of influenza bacilli from the nasopharyngeal secretion, volunteers 1, 2 and 3 were treated twice daily with a spray of dichloramin-T in chlorinated eucalyptol, and 2 days later the other two volunteers were treated in the same way in an effort to clear their throats. A few days later they were discharged from the hospital, but influenza bacilli were still present and even in greater numbers at times, although the number and types of other organisms were appreciably diminished, in some instances to such an extent that the influenza bacilli were present apparently in pure culture. This persistence of the influenza bacillus in the presence of such a powerful disinfecting substance as dichloramin-T is in accord with the difficulty some clinicians have noted in ridding convalescent influenza patients of this organism.

Following his discharge, one of the volunteers (6) used chlorazene for several days, resulting in the disappearance of the influenza bacilli. Three of the volunteers subsequently presented negative cultures, but one (1) still showed many colonies of typical influenza bacilli.

In all of the experiments the general condition of the men was apparently unaffected by the application of bacterial free filtrates of influenza material, or by the suspension of the various strains of influenza bacilli. In no case was there the least symptoms of influenza. The white blood cell count tended to be low, but not different from the period before the inoculation. The differential count showed nothing unusual.

The freshly isolated strain produced no more effect than the older strains. The filtrate experiment cannot be considered conclusive because it does not include enough cases; the filtrate was too old and material should have been taken earlier in the course of the disease, when the virus, if such is the case, would be more virulent. Some doubt is cast on the conclusions reached by Nicolle as to the existence of a virus.

The fact that the volunteers received such massive doses of influenza bacilli, much larger than could possibly happen through natural means, with no symptoms, makes it very doubtful if this organism alone can cause this widespread epidemic. To be sure, the fact that some of the volunteers were vaccinated against influenza vitiates the value of these negative results somewhat, yet 5 who had not been vaccinated, behaved in the same way and then, too, the efficacy and protective power of the vaccine is very much in doubt. Culture 69 was taken from a fatal case of influenza, the patient receiving the same vaccine that the volunteers did, about a month before his death.

CONCLUSIONS

The nasal application of a filtrate from a pneumonic lung of an individual dead from typical influenza bronchopneumonia failed to call forth any abnormal symptoms.

The application to the mucous membrane of the nares and nasopharynx of five healthy men (four inoculated from 4-6 weeks ago against influenza with a polyvalent influenza vaccine, one uninoculated) of freshly prepared suspension of four different live strains of B. influenzae (one, in the second generation from the fatally infected human host) even in the massive doses, failed to produce any abnormal symptoms.

The implantation of living suspensions of influenza bacilli produced no material alteration besides the addition of the influenza bacillus itself.

When experimentally implanted the influenza bacillus disappears from the nares in a relatively short time, from 24-72 hours.

When experimentally introduced in the nasopharynx of man the influenza bacillus exists and multiplies for a considerable length of time, two weeks or more, and apparently shows considerable resistance to the action of dichloramin T.

In the examination of the nasopharyngeal secretions of patients suffering from infections diagnosed clinically as typical influenza and in tissues of the respiratory tract of patients who died of influenzal bronchopneumonia, it was found that the oleate plates frequently gave positive results when the blood and chocolate plates were negative and in addition, by inhibiting the growth of streptococci and pneumococci, greatly facilitated the isolation of the influenza bacillus. In every case the chocolate plate gave more information than the blood plate, which was useful only in picking pneumococci and making a hasty diagnosis of the type of streptococcus present.

It is recommended that in the routine bacteriologic examination of all suspected influenza cases, plain, infusion, chocolate and oleate or soap agar be used. The blood plate should not be discarded, because it gives information regarding the presence or absence of streptococci, and pneumococci, that are liable to be missed otherwise and that may play a not unimportant part either in a symbiotic rôle or as a complicating factor.