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Studies of the Etiology of Influenza.

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For many years the etiology of influenza has been the subject of an interesting and continuous discussion. A number of microscopically visible organisms have been assigned a certain causative relationship to this disease. The most important of these micro-organisms is *H. influenzae,* the significance of whose relationship to influenza was first brought out by Pfeiffer. This organism has continued to hold a prominent position on the stage of debate concerning the disease. Another question that has led to much discussion is the degree of uniformity of nature of the pathological and clinical entity described as influenza. Interest in this regard has largely centered upon the identity or non-identity of the so-called pandemic and inter-pandemic forms of the disease. Clinically, examples of the disease in pandemic and in inter-pandemic periods may resemble each other so closely as to be indistinguishable. Whether they are the same condition from an etiologic standpoint can not, however, as yet be answered.

In recent years, with increasing knowledge of filterable viruses, this group of micro-organisms has been prominently considered in discussions of the etiology of influenza. Some evidence[1-5] has already been brought out tending to implicate them in the disease and it is with this aspect of influenza that the present work is concerned.

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[1] Dujarric de la Riviere, M. R., *Comp. rend. Acad. des Sci.*, 1918, **167**, 606.

[2] Selter, H., *Deut. Med. Woch.*, 1918, **44**, 932.

[3] Nicolle, C., and Lebailly, C., *Ann. de l'inst. Past.*, 1919, **33**, 395.

[4] Yamanouchi, Sakakami, Iwushima, *Lancet*, 1919, **1**, 971.

[5] Long, P. H., Bliss, E. A., Carpenter, H. M., *J. Am. Med. Assn.*, 1931, **97**, 1122.

During an outbreak of influenza of the inter-pandemic type in the winter of 1930, an effort was made to communicate the disease to chimpanzees by means of bacteria-free filtrates of nasopharyngeal washings obtained from patients with typical attacks of the disease. The washings were obtained during the first 36 hours of the attack, passed rapidly through Seitz filters and inoculated intranasally in chimpanzees. In all, 8 animals were so treated. In no instance did any of the apes manifest either local respiratory symptoms or recognizable constitutional reaction of any kind. All of the chimpanzees inoculated were recovering or had recently recovered from typical attacks of the common cold so that susceptibility may have been low.

Shortly after this 2 human volunteers were placed in strict quarantine for a period of 4 days. At the expiration of this time nasopharyngeal washings were obtained from an individual with an attack of influenza of the prevailing type. The important symptoms and signs of this patient were: Temperature 101°F. by mouth, general malaise, cough, and nasal obstruction, leucocytes 7,300. The patient was confined to bed for 4 days.

Volunteer 25 was inoculated intranasally with 6 cc. of a Berkefeld N filtrate of the above washings. The filtrate was negative both aerobically and anaerobically for bacterial growth. Within 24 hours of inoculation this volunteer developed nasal obstruction, sneezing, slight nasal discharge and slight headache. Later there was red throat and a productive cough. There was no fever and no constitutional reaction. By the third day the symptoms had abated considerably and the volunteer was discharged from the hospital. He appeared to have had a mild but definite common cold. One day after discharge the symptoms reappeared in more severe form. The subject was readmitted to the hospital with acute pharyngitis, fever of 102°F., marked constitutional reaction, cough and muco-purulent sputum. The manifestations lasted for about 4 days.

Volunteer 26 was inoculated with the same material and in a manner similar to Volunteer 25. This volunteer experienced no symptoms of respiratory infection either during his stay or after discharge from the hospital.

During an outbreak of influenza in February, 1932, a culture was made, in chick embryo medium, of filtered bacteria-free nasopharyngeal washings from an acute case of influenza. The technique employed was that used by us in the cultivation of the virus of the common cold.[6] The patient's symptoms were typical except for a

[6] Dochez, A. R., Mills, Katherine C., and Kneeland, Yale, Jr., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 513.

mild cystitis. There were two slight chills, the temperature reached 103°F., moderate cyanosis was present, mild conjunctivitis, running nose and slight sore throat. The leucocytes were at first 7,000 and later dropped to 4,840. The duration of the fever was 4 days.

This culture F. was carried in chick embryo medium, transferring every 3 or 4 days for 19 generations, a period of about 10 weeks. The F 19 generation was inoculated intranasally into Volunteers 55 and 56, under strict isolation. Volunteer 55 developed slight malaise and headache within 24 hours of the time of inoculation. Later there developed sneezing; nasal discharge; productive cough; red, sore throat; and moderate constitutional reaction. There was no fever. The white blood cells were 9,500. This individual experienced the symptoms of an acute common cold of marked severity.

Volunteer 56 developed symptoms synchronously with those of Volunteer 55. There was moderate constitutional reaction and very marked manifestations of respiratory tract inflammation. There was no fever and the white blood count was 10,650.

Both of these individuals experienced the symptomatology of the common cold. The attacks were of marked severity and acute signs of infection lasted for 7 days. Three weeks later the same 2 volunteers were re-inoculated with the F 25 generation of the culture in order to test for the presence of immunity. Both individuals again experienced attacks resembling the common cold, but of less severity than the preceding attacks.

Following these inoculations 5 more volunteers were inoculated and of these 4 experienced symptoms of a mild common cold and one, no reaction whatever. During the summer of 1932 the culture was transferred to London and in collaboration with C. H. Andrewes of the National Institute for Medical Research, 17 volunteers were inoculated. Of these 15 experienced no symptoms whatever. Two developed infections resembling the common cold, one having a moderate constitutional reaction and a temperature of 99.2°F. Inasmuch as isolation was not practiced and colds were prevalent at the time, reliable conclusions could not be drawn from the positive instances of infection.

The culture was brought back to the United States and has since been carried continuously in chick embryo medium. Generations F 57, F 65, F 80 and F 83 have been inoculated into human volunteers. Generation F 57 was inoculated into 3 individuals and induced mild symptoms of upper respiratory inflammation of the type of the common cold. Generation F 65 was inoculated also into 3

subjects, all of whom developed symptoms of upper respiratory infection within 36 hours after inoculation. The symptomatology in these individuals showed a change from that previously observed in the volunteers inoculated with cultures of typical common cold virus. The upper respiratory symptoms were relatively slight and consisted of sneezing, moderate nasal discharge and cough and mild sore throat. On the other hand, the constitutional reaction consisting of headaches, anorexia, general malaise with aching pains in the back and legs and a rise in temperature up to 1°F. was more pronounced than we have as yet observed in any individuals experimentally infected with tissue medium cultures from examples of acute upper respiratory infection. Generations F 80 and F 83 were each inoculated into 2 volunteers. These 4 individuals suffered from acute upper respiratory infections of the type described above, manifesting slight signs of respiratory tract irritation, constitutional reaction of the type just described and very slight fever. The duration of the symptoms ranged from 3 to 5 days.

Chimpanzees have been relatively insusceptible to experimental infection with the tissue culture virus of the common cold and with that cultivated from influenza. However, one of these animals has been successfully inoculated with Culture F 63. The animal had experienced no respiratory infection for 5 months. It was placed under strict quarantine and after a fore-period of observation was inoculated intra-nasally with generation F 63. Twenty-four hours later nasal obstruction and discharge appeared. For 4 days thereafter there were manifest definite but mild symptoms of the common cold. There was no fever and no prostration. Another animal inoculated at the same time with the same material exhibited no symptoms.

Culture F, isolated from the naso-pharynx of an individual suffering from clinical influenza, has over a period of 11 months produced experimental upper respiratory infections in a series of human volunteers. The character of the infection has been of 2 types, the first resembling the common cold with conspicuous signs of irritation of the respiratory tract, little constitutional reaction and no fever; the second somewhat different with slight signs of respiratory inflammation, moderate constitutional reaction and slight fever, never above 1°F.

In January, 1933, a second chick embryo medium culture was made of filtered bacteria-free, naso-pharyngeal washings from an acute case of influenza. There was at this time a rather wide-

spread outbreak of typical clinical influenza. This patient exhibited the usual symptoms, the temperature at the time the culture was made was 101.6° F., and the leucocytes were 5,800. The manifestations of respiratory irritation were slight and there were no complications.

This culture was carried in chick embryo medium, using the technique previously described, for 2 months when generation T 18 was inoculated intranasally into 2 human volunteers under strict isolation. Both of these individuals became infected and exhibited a similar symptomatology. Signs of infection appeared at the end of 24 hours and were still present at the time of discharge from quarantine 7 days later. The symptoms consisted of nasal obstruction, sneezing, marked nasal discharge, cough with the production of muco-purulent sputum, and redness and slight edema of the throat. The constitutional reaction was very slight and there was no rise in temperature. These 2 experimental infections resembled severe examples of the common cold and were very much like the first series of infections produced by inoculation of culture F 19 described above. Control inoculation of the same individuals, before experimental infection, with filtered naso-pharyngeal washings from a normal human being and with incubated sterile tissue culture medium have been negative except for moderate signs of temporary irritation.

By employing the technique used by us for the cultivation of the virus of the common cold it has been possible to propagate in tissue culture medium a filtrable agent from 2 typical clinical examples of influenza. Human volunteers have been repeatedly infected by intranasal inoculations with this cultivated agent, in one instance after the agent had been carried for 11 months in the culture medium and when it had reached the eighty-third generation. The symptomatology of the experimental infections in general has resembled that of the common cold. The respiratory infections induced have been somewhat more severe in character than those which have developed after inoculations with the cultivated virus of the common cold. Following inoculation with generations F 65, F 80, and F 83 the infected volunteers showed, in addition to the ordinary symptoms of the common cold, moderate constitutional reaction and slight fever. We believe that these experiments indicate that a filtrable agent has been cultivated from patients suffering from influenza. We have not as yet been able to distinguish this agent from that of the common cold. Because of the short duration of immunity in human beings, sometimes not more than 3

weeks, it has not been possible to perform experiments designed to bring out the immunological relationship between the agent described and that of the common cold.

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A Simply Prepared Broth for Producing Hemolytic Streptococcal Hematoxin (Streptolysin).*

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Todd[1] has demonstrated recently that the sera of patients suffering from various hemolytic streptococcal infections contain antibodies that neutralize streptococcal hematoxin, and he, collaborating with Coburn[1, 2] has shown that these antibodies are almost constantly present in abnormal concentrations in the serum of patients suffering from rheumatic fever. Obviously a new technique has been developed for the study of infections of this type. Todd's[3] original medium for the production of streptolysin consisted of horse meat infusion, peptone and yeast extract, which after mixing was sterilized by filtering, first through a coarse earth filter and then through Chamberland "F" candles. Later Todd and Hewitt[4] found that a more suitable medium could be prepared by substituting for the yeast extract the following substances: sodium bicarbonate as a buffer, inorganic phosphates and glucose as growth stimulating materials and sodium chloride to supply suitable isotonicity.

We have had little success with the broth containing yeast extract, but have obtained a satisfactory yield of streptolysin from the buffered horse meat broth, and a still better yield when fresh beef heart was substituted for the horse meat. The tedious and time-consuming filtration of the broth, through 2 different filters, makes the production of this medium very difficult unless special apparatus is available. It is possible, on the other hand, to prepare

* The generic term *hematoxin* is used for bacterially produced substances that lyse red blood cells, and the specific term *streptolysin* for such substances elaborated by hemolytic streptococci.

1 Todd, E. W., *Brit. J. Exp. Path.*, 1932, **13**, 248.

2 Coburn, A. F., and Pauli, R. H., *J. Exp. Med.*, 1932, **56**, 651.

3 Todd, E. W., *J. Exp. Med.*, 1932, **55**, 267.

4 Todd, E. W., and Hewitt, L. F., *J. Path. and Bact.*, 1932, **35**, 973.