

**STUDIES ON THE RELATION OF PNEUMO-  
TROPIC STREPTOCOCCI TO  
INFLUENZA VIRUS<sup>1</sup>**

SEROLOGICALLY distinct pneumotropic streptococci, which were used in these experiments, were isolated in studies of influenza and other respiratory infections in our brain-containing mediums, dextrose-brain broth and soft dextrose-brain agar (0.2 per cent. dextrose and 0.2 per cent. agar). These mediums are highly favorable for the isolation of specific types of streptococci and for obtaining pure cultures without loss of specificity.

White mice were inoculated intranasally with these streptococci, which had been subjected to one or more serial dilution cultures alternately in dextrose-brain broth and dextrose-brain agar.<sup>2</sup> The streptococci that grew at the end point of growth were used. All streptococci inoculated were so far removed from the original source that the possibility of passive transfer of "natural" influenza virus was eliminated. By serial intranasal passage, through mice and embryonated chicken eggs, of emulsions and filtrates of emulsions of pneumonic lungs thus obtained, and of allantoic fluid of infected embryonated eggs, a pneumotropic, filtrable infectious agent, transmissible in series, was obtained. The filtrable infectious agent was obtained from each of twenty-nine cultures of pneumotropic streptococci: fourteen cultures from the nasopharynxes or blood of thirteen persons having acute epidemic influenza, eight cultures of streptococci from a milk supply and two from a strain isolated from freshly fallen snow during epidemics of influenza and five strains isolated by me from "natural" influenza virus which had been sent to me for study.

<sup>1</sup> Preliminary report.

<sup>2</sup> E. C. Rosenow, *Arch. Path.*, 26: 70, 1938.

Under conditions employed successfully by others, influenza virus was obtained, by intranasal inoculation of mice, from filtrates of nasopharyngeal washings of six out of thirty patients during the acute stage of influenza.

Each of the strains of the filtrable infectious agent obtained from twenty-nine cultures of pneumotropic streptococci has been passed successively through from six to eighteen serial passages. Lesions of lungs occurred in altogether 1,130 (57 per cent.) of 1,900 mice inoculated with test material.

After a number of serial intranasal passages of the filtrable infectious agent obtained from pneumotropic streptococci, the incidence, type and degree of gross and microscopic lesions that developed in the lungs of mice were essentially the same as the incidence, type and degree of those that developed after intranasal inoculation of "natural" influenza virus. The incidence of isolation of streptococci from pneumonic lungs of mice that had received the experimental infectious agent and those that had received "natural" influenza virus also was similar. Isolations of streptococci and incidence of lesions, especially in the first number of serial passages, often ran parallel but, in general, isolations of streptococci diminished progressively with serial passages.

Strains of streptococci isolated from pneumonic lungs in the two groups of mice, those receiving the experimental infectious agent and those receiving "natural" influenza virus, had moderate pneumotropic virulence. Five strains of streptococci from the latter group, far removed from virus, yielded the infectious agent on successive passage of lung material, beginning with the streptococcus. The streptococci from both groups were agglutinated specifically by the influenza antistreptococcal serum and by convalescent influenza serum.

The infectious agent produced from streptococci

was as filtrable as "natural" influenza virus and remained viable on preservation in 50 per cent. glycerin for as long as three months.

The invasive power of both the experimental infectious agent and virus and of the influenzal type of streptococcus was neutralized by the influenza anti-streptococcal serum and by convalescent influenza serum but not by normal horse serum or normal human serum.

Mice that were immunized intranasally or intraperitoneally with vaccines prepared from freshly isolated streptococci that had been isolated from nasopharynges or blood of persons having symptoms of acute influenza became resistant to intranasal inoculation of the experimental infectious agent and "natural" influenza virus. Hence, the use of both streptococcal and viral vaccines in prophylaxis should afford protection.

The possibility that the infectious agent obtained in these experiments might represent pickups of latent or spontaneous pneumotropic virus, described by others,<sup>3-7</sup> in mouse stocks was considered and a report has been withheld until the evidence against such possibility seemed conclusive. Since the different strains of the infectious agent obtained by me from pneumotropic streptococci and "natural" influenza virus isolated by others are alike, the possibility of pickups of the latent virus in mice applies equally to my experiments and to the isolation and propagation in mice of "natural" influenza virus by others. The controls in both instances suffice, it would seem, to eliminate this possibility. Control inoculations made in 712 mice

<sup>3</sup> A. R. Dochez, K. C. Mills and B. Mulliken, *Proc. Soc. Exp. Biol. and Med.*, 36: 683, 1937.

<sup>4</sup> F. B. Gordon, Gustave Freeman and J. Marion Clam-  
pit, *Proc. Soc. Exp. Biol. and Med.*, 39: 450, 1938.

<sup>5</sup> F. B. Gordon and Helen V. Karr, *Proc. Inst. Med. Chicago*, 14: 370, 1943.

<sup>6</sup> Helen V. Karr, *Jour. Infect. Dis.*, 72: 108, 1943.

<sup>7</sup> Clara Nigg, *SCIENCE*, n.s., 95: 49, 1942.

during the course of these experiments with (1) emulsions or filtrates of emulsions of lungs of normal mice and of the few uninoculated mice in which lesions of lungs had been found, which, however, clearly were different from those in test mice; (2) filtrates of dextrose-brain broth cultures of the streptococcus, and (3) filtrates of dextrose-brain broth and chick-embryo medium, and inoculations made in 1,142 mice with non-pneumotropic streptococci from sources remote from influenza, did not yield the infectious agent.

The data obtained indicate that the pneumotropic, filtrable, transmissible infectious agent obtained from pneumotropic streptococci appears to be true influenza virus, as now understood, and that pneumotropic streptococci in influenza and related respiratory infections, such as primary atypical pneumonia and influenzal bronchopneumonia, may be an important source of what is now considered virus.

EDWARD C. ROSENOW,  
*Now Professor Emeritus*

DIVISION OF EXPERIMENTAL BACTERIOLOGY,  
MAYO FOUNDATION,  
ROCHESTER, MINN.