having gonorrhea and one urethritis with extracellular diplococci.

(2) There was one failure, Subject No. 10, who received only 30,000 units.

(3) Eight of the patients with gonorrhea and the one patient with urethritis were cured (bacteriologically and clinically) by this single intramuscular injection of penicillin.[3]

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STUDIES ON THE RELATION OF PNEUMOTROPIC STREPTOCOCCI TO INFLUENZA VIRUS[1]

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.[2] 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 nasopharynges 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.

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 antistreptococcic 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 antistreptococcic 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

[3] The cooperation of Commander G. J. Thompson (M.C.), U.S.N.R., chief of the Urological Service, and Lieutenant P. V. Wooley, Jr., officer in charge of the Bacteriological Laboratory, is appreciated. The blood assays were made by Barbara C. Unsworth, PhM1c, and W. E. Lenert, PhM3c, using a serial dilution method. The sketch of the ice-bag harness was made by J. DiFerdinando, PhM3c, S-V, U.S.N.R.

[1] Preliminary report.

[2] E. C. Rosenow, *Arch. Path.*, 26: 70, 1938.

acute influenza became resistant to intranasal inoculation of the experimental infectious agent and "natural" influenza virus. Hence, the use of both streptococcic 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,[3-7] 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 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.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

SCREW-CAPPED BACTERIOLOGICAL CULTURE TUBES

A NEW screw-capped bacteriological test-tube has been placed on the market by the Will Corporation.[1] This test-tube has been tried in our laboratory and has been found to have certain advantages over the conventional cotton-plugged culture tube. For example, media that need to be made up only occasionally can be kept in the screw-capped tubes fresh and moist at room temperature for many months. The experience with this new tube has shown, however, that it still could be improved considerably by extending the length of the screw cap and the neck of the tube from the present length of about 10 mm to the least 25 mm without changing the thread.

Fig. 1 shows the suggested shape and length of the neck with the screw thread and the cap. Three important improvements could be achieved by such modification of design. (1) Tubes with long caps and necks can be incubated with the caps slightly unscrewed to permit the same free exchange of air as through the usual cotton plugs, without any increased danger of contamination, whereas with the short caps contamination occurs readily if the caps are not closed tightly. (2) Since the present short caps must be closed tightly immediately after the removal of the test-tubes from the sterilizer, because otherwise they do not protect the medium sufficiently from contamination during the process of cooling, a negative pressure develops in the cooled-off tubes. When such

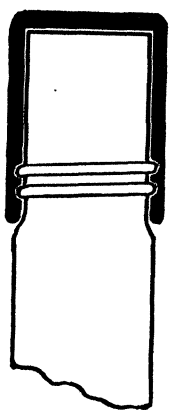

FIG. 1

partly evacuated tubes are opened for inoculation, the air current entering the tubes may sometimes introduce contaminating microorganisms. Also, the short cap will stay in place only when screwed on so far that the vinylite lining of the cap sticks to the end of the tube, whereupon inrushing air forces the lining suddenly down into the tube. By having the necks and the screw caps much longer than the present ones,

[3] A. R. Dochez, K. C. Mills and B. Mulliken, *Proc. Soc. Exp. Biol. and Med.*, 36: 683, 1937.
[4] F. B. Gordon, Gustave Freeman and J. Marion Clampit, *Proc. Soc. Exp. Biol. and Med.*, 39: 450, 1938.
[5] F. B. Gordon and Helen V. Karr, *Proc. Inst. Med. Chicago*, 14: 370, 1943.
[6] Helen V. Karr, *Jour. Infect. Dis.*, 72: 108, 1943.
[7] Clara Nigg, SCIENCE, n.s., 95: 49, 1942.
[1] Catalog Supplement Number Three, p. 21, No. 16535.