other sections of the community, a more exact estimate of the carrier rate and the geographic and seasonal distributions can be made.

## 8016 C

## Immunological Relationships of Strains of Filtrable Virus Recovered from Cases of Human Influenza.

THOMAS FRANCIS, JR. (Introduced by Rufus Cole.)

*From the Hospital of the Rockefeller Institute, New York.*

Smith, Andrewes and Laidlaw[1] isolated a filtrable virus from the nasopharyngeal washings of influenza patients following the inoculation of these materials into ferrets. Two strains of virus isolated by them in England during successive winters were found to be immunologically identical.[2] These same workers reported that the virus of swine influenza isolated by Shope[3] was antigenically related to the human strains.

During the Autumn of 1934, at the Hospital of the Rockefeller Institute, we were successful in infecting ferrets and mice with strains of a filtrable virus obtained from the sputum of cases of epidemic influenza in Puerto Rico.[4] These 2 strains have been called P. R. 5 and P. R. 8. Additional strains of virus have been isolated from cases of influenza in New York and Philadelphia. During the course of these experiments, Andrewes, Laidlaw and Smith[2] reported independently that they had successfully infected mice with the viruses of both swine and human influenza. They also reported that the serum of a hyperimmune horse, or of hyperimmune ferrets, neutralized the infectivity of the respective strains of virus.

The infection in mice, following the intranasal inoculation of the virus is characterized by the development of pulmonary lesions, but death of the animals is somewhat irregular. The serum of ferrets recovered from infection, when mixed with suspensions of the homologous strain of virus and instilled into the nasal passages of mice, has been found to prevent the development of these pulmonary lesions. The serum of normal ferrets, however, has no neutralizing

1 Smith, W., Andrewes, C. H., and Laidlaw, P. P., *Lancet*, 1933, **2**, 66.

2 Andrewes, C. H., Laidlaw, P. P., and Smith, W., *Lancet*, 1934, **2**, 859.

3 Shope, R. E., *J. Exp. Med.*, 1931, **54**, 373.

4 Francis, T., Jr., *Science*, 1934, **80**, 457.

TABLE I.
Protection of Mice Against Influenza Virus.

| Strain of Virus | | Severity of Pulmonary Lesions | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | P.R.8 Strain | | | | | Phila. Strain | | | | |
| Mouse No. | | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| Serum: | | | | | | | | | | | |
| Ferret P.R.8 | 1:2 | 0 | 0 | 0 | 0 | 0 | ± | 0 | 0 | 0 | 0 |
| | 1:10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 |
| Ferret Phila. | 1:2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1:10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ferret P.R.5 | 1:2 | 0 | 0 | 0 | 0 | 0 | | | | | |
| Ferret Normal | 1:2 | +++ | +++ | ++++ | +++ | ++++ | ++++ | +++ | ++++ | ++++ | ++++ |
| Immune Horse* | 1:2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ± | 0 | 0 |
| | 1:10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Normal Horse | 1:2 | ++++ | ++++ | ++ | +++ | ++++ | ++++ | ++++ | +++ | ++++ | +++ |
| Anti-Swine Influenza (Shope) | 1:2 | +++ | ++++ | ++++ | +++ | ++++ | | | | | |

0 = No pulmonary involvement.
± to ++++ = Increasing degrees of pulmonary involvement.
* This serum was obtained through the courtesy of Dr. Andrewes, of the National Institute for Medical Research, London, England.

effect. It is possible, therefore, to measure in mice the neutralizing capacity of the serum of a recovered ferret against homologous and heterologous strains of virus.

The sera available were those of ferrets recovered from infection with one or another of 3 strains of human influenza virus: P. R. 5 strain, P. R. 8 strain, or Philadelphia strain; the serum of a horse immunized by Andrewes, Laidlaw and Smith against the British (W.S.) strain of influenza virus; and the serum, obtained from Dr. Shope, of swine convalescent from infection with Shope's swine influenza virus.

The tests are performed as follows: Weighed amounts of infected mouse lung, as the source of virus, are ground with physiological salt solution to form a 10% suspension. After centrifugation of the suspension for 15 minutes, equal portions of the virus suspension and serum are mixed and incubated at 37°C. for 30 minutes. Each of 5 mice, lightly anesthetized with ether, is then inoculated intranasally with 0.03 cc. of the mixture. On the 5th or 6th day after inoculation, at which time the control mice are dying, all other mice are killed and their lungs removed. The effect of sera of unknown potency upon the infectivity of the virus is measured by comparing the severity of the pulmonary lesions in mice receiving mixtures of those sera and virus, with the lesions in control mice receiving virus and normal ferret or horse serum.

The results of a typical protective experiment are presented in Table I.

It was found that the serum of ferrets recovered from infection with the Puerto Rico and Philadelphia strains reciprocally neutralized both strains of virus (P. R. 8 and Philadelphia), whereas normal ferret serum had no effect. Immune horse serum, prepared by Andrewes and his coworkers against the British strain of virus, also neutralized completely these 2 strains of virus. Serum of swine convalescent from infection with the virus of swine influenza, and known to neutralize swine influenza virus failed to neutralize strains of human influenza virus. These results indicate that the strains of virus recovered from cases of human influenza in Puerto Rico, Philadelphia and England are immunologically identical, whereas the virus of swine influenza differs serologically.

Furthermore, it was found that the serum of human individuals in New York, taken during convalescence from influenza, gave marked protection to mice against the Puerto Rico strain of virus, while the serum of the same individuals taken during the acute stage of the disease did not.

It appears, therefore, that the virus obtained from human influenza is a distinct entity and is etiologically related to the human disease.

## 8017 P

## A Method for Titrating the Protective Action of Antimeningococcal Serum.

GEOFFREY RAKE.

*From the Laboratories of The Rockefeller Institute for Medical Research, New York City.*

Miller[1] has described a method for the production of experimental meningococcal infection in mice. It consisted in brief of the use of a 6% mucin suspension buffered at 7.4 as a medium in which the organisms were suspended prior to intraperitoneal inoculation. More recently Miller has modified the technique of preparing the mucin.[2] A 5% suspension is now prepared, it is sterilized in the autoclave at 10-15 lb. pressure for 15 minutes, sterile dextrose solution is then added to a final concentration of 1%, and the pH adjusted to pH 7.4 with sterile buffer solution.

Using such a mucin suspension, the intraperitoneal virulence of meningococcus strains can be titrated,[1, 3] and consistent results will be obtained when pure breeds of susceptible mice are employed. It has been found, in accord with Miller's work, that freshly isolated strains may kill when the cultures are diluted as far as $10^{-8}$, that is approximately 20 organisms. A brief report has been made elsewhere[3] on the application of this experimental meningococcal infection to the test of sera for their protective activity. That report dealt chiefly with the content of protective antibodies in the serum of carriers of the meningococcus and in the serum of normal individuals. The high protective value of some antimeningococcal sera was demonstrated but no titration was carried out.

In testing the intraperitoneal virulence of strains, 14 to 18-hour cultures on 10% rabbit's blood pneumococcus agar plates are washed off with normal saline and the suspension is diluted in saline and adjusted with a Gates turbidometer to the standard of 2,000,000,000 organisms per cc. Serial dilutions 1:10 are made in mucin and

[1] Miller, C. P., *Science*, 1933, **78**, 340.

[2] Miller, C. P., personal communication.

[3] Rake, G., *J. Exp. Med.*, 1935, **61**, 545.