antibodies (anti-Rh_o) which were active with Rh_2 cells were demonstrated in antiserums containing agglutinins (anti-Rh′) for a different Rh subtype (Rh_1). This observation suggests further study on the specificity and origin of blocking antibodies in respect to subtypes of Rh agglutinogen.*

* Since the completion of this study our attention has been called to a publication by Race[3] in which blocking antibodies of standard anti-Rh specificity were demonstrated in anti-Rh′ serums. This observation was also made by Wiener[4] whose findings are awaiting publication.

[3] Race, R. R., *Nature*, 1944, **153**, 771.

[4] Wiener, A. S., *Science*, in press.

14847 P

The Toxic Effect of Influenza Virus in the Rabbit Eye.*

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Injection of type A and B influenza virus into the rabbit eye has been found to cause corneal opacity without multiplication of the virus.

Materials and Methods. The PR8 strain of influenza type A and the Lee strain of influenza type B virus were used in the form of allantoic fluid collected from infected chick embryos 48 hours after inoculation and preserved by freezing on CO_2 ice. The 50% mortality[1] titer of the PR8[2] strain in mice was $10^{-5.5}$ and the 50% infectivity titer in eggs[3] was $10^{-8.0}$ The corresponding titers for the Lee[4] strain were $10^{-3.7}$ in mice and $10^{-7.8}$ in eggs. Specific antisera were obtained from ferrets 10 days after intranasal inoculation with either the PR8 or the Lee strain of virus.

Young adult domestic rabbits, in nearly all instances of the albino variety, were inoculated under ether anesthesia by insertion of a 26-gauge needle through the cornea near the limbus. After withdrawal of slightly more than 0.2 cc of aqueous humor, approximately 0.2 cc of the inoculum was injected.

Observations. In the course of several experiments 15 rabbit eyes were inoculated with the PR8 virus and 17 with the Lee virus as described. Without exception there developed a haziness of the cornea which progressed to a complete opacity. As a rule the cornea remained clear for 48 hours, except for the transient alteration due to trauma at the point of inoculation. The reaction was definite on the third day and attained a maximum on the fourth or fifth day, at which time the cornea was thickened and obviously edematous. In a few instances, the reaction was more rapid, some haziness being apparent at 24 hours and the opacity being complete at 72 hours. With the exception of moderate conjunctival injection and congestion of the iris, lesions were not noted in other parts of the eye than the cornea.

In 10 eyes inoculated with normal allantoic fluid and 4 with mixtures of normal allantoic fluid and normal or immune ferret sera no lesions were observed. Infected allantoic fluid inactivated by formalin 1:1000 for 12 hours or by temperature of 56°C for five minutes did not produce lesions. Likewise Seitz filtrates of infected fluids caused no pathological changes.

Lesions were only produced by the inoculation of large amounts of active virus. When the inocula were diluted 10 times in horse serum-broth typical opacity resulted; how-

* This work was supported by funds from the International Health Division of the Rockefeller Foundation, the John and Mary R. Markle Foundation, and the Graduate School of the University of Minnesota.

[1] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **38**, 59.

[2] Francis, T., Jr., *Science*, 1934, **80**, 457.

[3] Hirst, G. K,. *J. Immunol.*, 1942, **48**, 285.

[4] Francis, T., Jr., *Science*, 1940, **92**, 405.

ever, on diluting the inocula 100 times the eyes remained normal.

Neutralization tests were carried out by injecting a mixture of equal parts of undiluted serum and virus into the rabbit eye. Homologous antiserum from convalescent ferrets afforded complete protection. Normal ferret serum and heterologous influenza antiserum, type A and B, failed to protect the eye.

Tests for the persistence or multiplication of virus in the eye were carried out at intervals ranging from 8 hours to 4 days after inoculation. Aqueous humor or a suspension of tissues from the anterior portion of the eye were injected into 6-11-day-old chick embryos in each test. The results of two experiments showed that the virus disappeared quickly from the aqueous humor. Tissues from the anterior portion of the eye retained small amounts of virus (end points 10^{-2} or 10^{-3}) for three days. On the fourth day the virus was practically absent as only one out of 12 eggs injected with a 10^{-1} dilution of ocular tissues was infected.

Discussion. Inasmuch as there appeared to be a complete lack of multiplication of influenza virus in the eyes of rabbits, the severe corneal damage was attributed to a toxic effect and not to infection. The type specificity of the neutralizing power of antiserum shows that the toxic substance is not a nonspecific irritant in the inoculum but must be either the virus particle itself or some other antigenic substance generated by the virus in the infected ferrets as well as in chick embryos.

The recent reports of Rake and Jones[5] have shown a toxic effect of the viruses of lymphogranuloma venereum, mouse pneumonitis, and meningo-pneumonitis. It would appear that the corneal opacity observed in these experiments and neurologic disturbances observed by the Henles in mice[6] are probably caused by toxic properties of the influenza viruses.

5 Rake, G., and Jones, H. T., *J. Exp. Med.*, 1944, **79**, 463.

6 Henle, G., and Henle, H., *Science*, 1944, **100**, 410.

14848

Prolongation of the Action of Penicillin After Intramuscular Injection.*

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Penicillin is so rapidly excreted from the body after parenteral injection that frequent administrations are necessary to maintain it continuously in the blood in measurable amounts. Although intermittent therapy may often be adequate, particularly when this involves spikes of high penicillin concentrations immediately after the injections, it is probable that a long high plateau is still more desirable. To this end, several procedures have been devised to delay the excretion, or retard the absorption, of penicillin after injection.

Rammelkamp and Bradley[1] and Beyer and co-workers,[2] using diodrast or para-aminohippuric acid to interfere with renal function, succeeded in depressing the excretion of penicillin in the urine of men and dogs. So far, this rather unphysiological method has apparently not received adequate clinical trial.

Raiziss[3] found that, when penicillin was injected intramuscularly into rabbits as a suspension in a vegetable oil, the blood remained bacteriostatic considerably longer

* Supported by the Rockefeller Fluid Research Fund and by the Winthrop Fellowship in Pharmacology.

1 Rammelkamp, C. H., and Bradley, S. E., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 30.

2 Beyer, K. H., Woodward, R., Peters, L., Verwey, W. F., and Mattis, P. A., *Science*, 1944, **100**, 107.

3 Raiziss, G. W., *Science*, 1944, **100**, 412.