in the determination of sex[5] until further study assures us that the *cyclical* appearance of a positive reaction is unquestionably limited to females.

[1] Loewe, S., *Klin. Wchnschr.*, 1925, iv, 1407.

[2] Frank, R. T., Frank, M. L., Gustavson, R. G., and Weyerts, W. W., *J. Am. Med. Assn.*, 1925, lxxxv, 510.

[3] Allen, E., and Doisy, E. A., *J. Am. Med. Assn.*, 1923, lxxxi, 819.

[4] Frank, R. T., and Goldberger, M. A., *J. Am. Med. Assn.*, 1926, lxxxvii, 1719; *J. Am. Med. Assn.*, 1928, xc, 106; *J. Am. Med. Assn.*, 1928, (February 4).

[5] Frank, R. T., and Goldberger, M. A., *J. Am. Med. Assn.*, 1926, lxxxvii, 554.

[6] See 2; also Frank, R. T., Gustavson, R. G., Holloway, J., Hyndeman, D., Kreuger, H., and White, J., *Endocrinology*, 1926, x, 260.

[7] Dohrn, M., *Klin. Wchnschr.*, 1927, vi, 359.

[8] Hirsch, *Klin. Wchnschr.*, 1928, vii, 313.

3910

Immunity in Guinea Pigs to the Virus of Vesicular Stomatitis.

PERRIN H. LONG AND PETER K. OLITSKY.

From the Laboratories of the Rockefeller Institute for Medical Research.

It is known that the injection of immune serum into guinea pigs prevents generalization of the lesions but not the primary vesicles of foot-and-mouth disease. In studying a strain of the virus of vesicular stomatitis, a disease of horses closely related to foot-and-mouth disease of cattle,[1] we have found that the virus, when injected into guinea pigs, loses its original feeble power to produce the characteristic secondary lesions in the pad, and that only primary lesions arise after pad inoculation. Notwithstanding this fact, the virus receives a general distribution since it can be recovered, 48 hours after pad inoculation into guinea pigs, from the apparently normal tongue. On the other hand, when the virus is injected into the muscles or the skin (intradermal) elsewhere than in the pad, no local lesion whatever follows, and 10 days after the inoculation it is found that the pigs are immune to reinoculation.

In the preliminary experiments, no attempt was made to titrate the strength of the virus, because present methods are crude, and once the infectivity of a given sample of virus has been determined, it is impossible to estimate the rate of its deterioration. Guinea pig pad vesicle fluid, obtained 24 to 48 hours after inoculation, diluted 1:10 and 1:20 with phosphate buffer at a pH of 7.5; and filtered

through a Berkefeld "V" candle, was employed in the following experiments. The immune serum was obtained from guinea pigs 10 to 14 days after inoculation.

Neutralization of the virus *in vitro* was first undertaken. 1 cc. of a 1:10 dilution of the virus was added to 1 cc. of immune serum and the contents of the tubes thoroughly mixed. The tubes were kept at room temperature for one minute, one hour, and 24 hours respectively, at which times the virus-serum mixture was injected into the pads of normal guinea pigs. Normal guinea pig serum was used as a control. Lesions appeared only in the animals treated with normal serum. After 10 days the pigs inoculated with immune serum and virus were retested by pad inoculation. All developed typical lesions.

Next, the pads of 4 normal guinea pigs were infiltrated with immune serum and the pads of 2 with normal guinea pig serum. One hour later the pads of 2 of the guinea pigs treated with immune serum, and those of one pig with normal serum were injected with a 1:20 dilution of the virus. The remaining 3 pigs were similarly inoculated with a 1:20 dilution of the virus on the following day. Lesions appeared only in the pads infiltrated with normal serum. Ten days later, all animals were reinoculated in the pads with active virus, and only the immune serum group developed typical vesicles.

Following this experiment, we determined the protection given by the intramuscular injection of immune serum, succeeded by intracutaneous or intramuscular inoculation of the virus. 0.5 cc. of immune serum was injected into the right thigh muscles of 8 normal guinea pigs. One hour later 2 animals were injected in the pads with a 1:10 dilution, and 2 with 0.5 cc. of a 1:20 dilution of the virus in the left thigh muscles; 24 hours later this procedure was repeated with the 4 remaining guinea pigs. Typical lesions appeared in the pads of the 4 animals inoculated in this tissue, while those receiving the virus intramuscularly revealed no manifest lesions. On retesting 10 days later with active virus, the animals inoculated intramuscularly developed typical lesions.

The conclusions which we draw from these experiments are. (1) that the virus generalizes through the body, although it induces no visible changes; (2) that following single intramuscular or intracutaneous inoculation of living virus at sites other than the pads, although no manifest lesions occur, a solid immunity results; (3) that a certain degree of neutralization of vesicular stomatitis virus *in vitro* and *in vivo* results from the addition of the serum of im-

mune animals; (4) that no immunity results from the injection of neutralized virus.

[1] Olitsky, P. K., Traum, J., and Schoening, H. W., *J. Am. Vet. Med. Assn.*, 1926, lxx, 147; Olitsky, P. K., *J. Exp. Med.*, 1927, xlv, 969.

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Mechanism of the Inhibition of Bacteriophagy by Agar or Gelatin.

J. BRONFENBRENNER AND D. HETLER.

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

It has been stated that an increase in the concentration of gelatin or agar in the medium tends to inhibit the lysis of bacteria by bacteriophage. d'Herelle explains this action on the assumption that the excess of gelatin or agar inhibits the normal growth of *Bacteriophagum intestinale* by interfering with the free diffusion of the products of its metabolism.[1] This explanation is not acceptable so long as there exists no satisfactory evidence of the metabolic activity of bacteriophage. Our recent studies have shown that lysis of bacteria may be the direct result of rupture of the bacterial cells due to increased uptake of water from the medium.[2] If this is true, inhibition of lysis in the presence of high concentrations of agar or gelatin in the medium may result from a competition for water between the medium and the bacteria.

Petri plates containing nutrient medium of different concentrations of agar or gelatin were seeded with susceptible bacteria and subsequently minute droplets of bacteriophage were deposited at different places on the seeded surface. The plates were allowed to dry for one hour under porous clay covers both before and after deposition of phage, in order to prevent its spreading. Contact impressions were taken at regular intervals on coverslips from the spots on which phage was deposited.

Macroscopic observation of the plates showed that lysis of bacteria occurred only in the plates containing low concentrations of agar or gelatin (1 to 2% and 15 to 25% respectively). The plates containing 4% agar, as well as those containing 50% gelatin, showed, on the contrary, a marked increase in the density of bacterial growth on the spots where phage was deposited, from which it was concluded that phage exerted a stimulating effect on the