5138

Studies on the Nature of the Viricidal Antibodies in Anti-Poliomyelitis Serum.*

E. W. SCHULTZ, L. P. GEBHARDT AND L. T. BULLOCK.

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

That poliomyelitis convalescent serum, whether of human or monkey origin, possesses viricidal properties demonstrable *in vitro* is a well established fact (Levaditi and Landsteiner,[1] Flexner and Lewis,[2] Netter and Levaditi,[3] and many others). The nature of the antibodies responsible for the inactivation of the virus has not been determined. While the results of several investigators (Lebredo and Recio,[4] Neustaedter and Banzhaf[5]) suggest that they may be cytolytic or bactericidal (complement fixing) in nature, others (Wollstein,[6] Gay and Lucas,[7] Römer and Joseph[8]) have been entirely unable to elicit evidence of specific antibodies of this type. Because of the difference in the results reported, particularly when considered in the light of the ultrafiltrable nature of the virus (Krueger and Schultz[9]), a careful study has been undertaken to determine, if possible, the exact nature of the antibodies formed against this virus. The work has been carried out with both human and monkey convalescent serum.

No difficulty has been experienced in demonstrating viricidal antibodies in convalescent sera tested *in vitro* in the customary manner. *No evidence of the presence of specific complement fixing and precipitating antibodies for the virus could, however, be elicited in any of these sera.* These results harmonize with those in earlier work on vaccinia, rabies, herpes and the bacteriophage (Schultz *et al*[10]). To determine further the relationship of the antibodies responsible for

* These studies were supported by Mrs. John W. Mitchell and the Mary Hooper Somers Medical Research Fund.

1 Levaditi and Landsteiner, *Compt. rend. Soc. biol.*, 1910, **68**, 311.

2 Flexner and Lewis, *J. Am. Med. Assn.*, 1910, **54**, 1780.

3 Netter and Levaditi, *Compt. rend. Soc. biol.*, 1910, **68**, 617.

4 Lebredo and Recio, *Sanidad y Beneficiencia* (Habana), 1910, **3**, 170. Cited by Gay and Lucas, *Arch. Int. Med.*, 1910.

5 Neustaedter and Banzhaf, *J. Infect. Dis.*, 1917, **21**, 515.

6 Wollstein, *J. Exp. Med.*, 1908, **10**, 476.

7 Gay and Lucas, *Arch. Int. Med.*, 1910, **6**, 330.

8 Römer and Joseph, *Münch. med. Wochenschr.*, 1910, **57**, 945.

9 Krueger and Schultz, PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 600.

10 Schultz, *et al.*, *J. Immunol.*, 1928, **15**, 229; 243; 265; 411; 1929, **17**, 245.

the inactivation of the virus, tests were carried out to determine whether neutral serum-virus mixtures are *dissociable,* and whether *time* is a factor in the inactivation of virus by immune serum. The results have been in the affirmative.

Experiment 1. *To Determine Whether Neutral Serum-Virus Mixtures are Dissociable.*

Four monkeys were used. Two were injected with undiluted serum-virus mixture, administered after the usual 2-hour incubation at 37° C., while 2 received corresponding mixtures diluted with 9 volumes of physiological saline immediately before injection. One monkey in each set of 2 was injected with normal serum-virus mixtures, undiluted and diluted, for control purposes. The results show that a neutral serum-virus mixture may be rendered infective again by the addition of a suitable volume of physiological saline. These observations, which agree with those made on neutral toxin-antitoxin mixtures by other investigators (Madsen,[11] Otto and Sachs,[12] Glenny[13]) indicate clearly that the inactivation of poliomyelitis virus by immune serum resembles more closely a toxin-antitoxin reaction than a bactericidal type of reaction.

Experiment 2. *Influence of Time on the Neutralization of the Virus by Immune Serum.*

Three groups of 2 monkeys each were inoculated with serum-virus mixtures which had been incubated for different lengths of time. One monkey in each set, receiving normal serum-virus mixtures, served as controls. The first set of 2 animals received the serum-virus mixtures immediately after the 2 reagents were brought together, the second set of 2 animals received similar mixtures after these had been incubated at 37° C. for 24 hours and the third set of 2 received mixtures after 48 hours' incubation. Of these 6 animals, only the 2 receiving immune serum-virus mixtures incubated 24 and 48 hours respectively, escaped the experimental disease. These results seem to indicate that *time* plays an important rôle in the inactivation.

In carrying out this experiment 2 precautions were regarded essential, one relating to the dilution of the immune serum to be used, the other to the prevention of spontaneous deterioration of the virus incident to prolonged incubation at 37° C. That the dilution of an immune serum may conceivably influence the rate of inactivation seems obvious, though this is sometimes overlooked. A concentrated

[11] Madsen, *Centralbl. f. Bakt.*, 1906, Orig., **37**, 373.

[12] Otto and Sachs, *Z. f. Exp. Path.*, 1906, **3**, 19.

[13] Glenny, *J. Hyg.*, 1925, **24**, 301.

immune serum added to an appropriate quantity of virus may render such a virus suspension innocuous immediately after the serum is added. In harmony with the results of other investigators with certain other viruses, we have repeatedly injected monkeys with poliomyelitis virus treated with *undiluted* monkey poliomyelitis convalescent serum immediately before inoculation (that is, without any incubation of the mixtures) without realizing infection. These observations have given rise to the opinion that the immune sera against the viruses may not act directly on the viruses, but in some indirect manner change the susceptibility of the tissues of the host. However, the fact that with dilution of the immune serum, it becomes necessary to prolong its contact with the virus *in vitro* argues distinctly in favor of the opinion that the immune serum *does* act directly on the virus. The relationship between the factor of *time* and that of *dilution* is brought out clearly by Schultz, Quigley and Bullock[14] on the inactivation of bacteriophage. They noted that whereas a serum dilution of 1:128 served to inactivate a given bacteriophage suspension in 30 minutes, a serum dilution of 1:4098 required as long as 8 days to render the same bacteriophage suspension inactive. It appears safe to assume that essentially the same relationships may apply to poliomyelitis and other virus-serum mixtures.

5139

Immune Serum Production in Poliomyelitis Refractory Animals.*

E. W. SCHULTZ AND L. P. GEBHARDT.

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

Several investigators have reported the production of antipoliomyelitis serum in poliomyelitis refractory animals. Thus, Kraus[1] and likewise Pettit[2] claim to have been able to produce a viricidal serum in sheep, while more recently Neustaedter and Banzhaf[3]

[14] Schultz, Quigley and Bullock, *J. Immunol.*, 1929, **17**, 245.

* These studies were supported by Mrs. John W. Mitchell and the Mary Hooper Somers Medical Research Fund.

[1] Kraus, *Z. f. Immunitätsforsch. u. Exp. Therapie*, 1911, **9**, 117.

[2] Pettit, *Compt. rend. Soc. biol.*, 1918, **81**, 1087.

[3] Neustaedter and Banzhaf, *J. Am. Med. Assn.*, 1917, **68**, 1531.