

10396 P

Type "B" Anti-influenzal Rabbit Serum for Therapeutic Purposes.

HATTIE E. ALEXANDER. (Introduced by R. McIntosh.)

From the Department of Diseases of Children, College of Physicians and Surgeons, Columbia University, New York.

The treatment of influenzal meningitis in children with sera from horses immunized with *H. influenzae* has, in general, been unsuccessful.

The investigations of Goodner and Horsfall^{1, 2, 3} demonstrated the superiority of rabbit over horse antipneumococcal serum. The causes of this superiority were believed to be, first, the smaller size of the anticarbohydrate molecule of rabbit serum, endowing it with greater powers of penetration of the tissues, and secondly, the absence of the prozonal phenomenon⁴ or inhibitory mechanism in rabbit serum, when amounts greater than a critical quantity were used.

Rabbit anti-influenzal serum, because of its greater penetrating powers, and some evidence that it crossed the blood-brain barrier, seemed to offer theoretical advantages over horse serum, assuming that the important antibody of the anti-influenzal serum is the anticarbohydrate as in rabbit antipneumococcal serum.

Pittman⁵ has brought forth much evidence that there is a parallelism between the antigenic structure of pneumococci and *H. influenzae*. She demonstrated a capsule on all smooth strains by Muir's capsule-stain and showed that such strains had the power to form soluble type-specific substances. All strains were found to be bile-soluble and therefore apparently produced enzymes as does the pneumococcus.

Because of this similarity, the knowledge derived from the study of pneumococci has been applied in the selection of a suitable antigen for the production of rabbit antibodies for *H. influenzae*. The strains used were those isolated from cases of influenzal meningitis. All the meningeal strains from children thus far reported have been Type B. Typing was performed by the capsular-swelling

¹ Horsfall, F. L., Jr., Goodner, K., MacLeod, C. M., and Harris, A. H., 2nd, *J. A. M. A.*, 1937, **108**, 1483.

² Horsfall, F. L., Jr., Goodner, K., and MacLeod, C. M., *N. Y. State J. Med.*, 1938, **38**, 245.

³ Goodner, K., Horsfall, F. L., Jr., and Dubos, R. J., *J. Imm.*, 1937, **33**, 279.

⁴ Goodner, K., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1936, **64**, 369.

⁵ Pittman, M., *J. Exp. Med.*, 1931, **53**, 471.

method with antisera obtained from Pittman. A large loopful (3-4 mm) of Type B antiserum is placed upon a coverslip. To it is added a 1 mm loopful of dilute young culture or biological fluid containing the suspected *H. influenzae* organisms. With this a 1 mm loopful of Loeffler's methylene blue is thoroughly mixed. The resulting mixed drop is then suspended over a hollow-ground slide and sealed with cedar oil. The capsules when exposed to type-specific antisera become swollen, opaque, and outlined as do pneumococci typed by the Neufeld method. This hitherto unreported procedure has been useful in the rapid identification of *H. influenzae* before cultural results are available, and in the determination of the stage at which cultures are presumably most suitable for use as antigen.

For the latter purpose, we have seeded Levinthal broth with 15% of its volume of a 24-hour broth culture, incubated 5 to 6 hours, added formalin to 0.5% and centrifuged, resuspending the organisms in 1/10 the original volume of physiological saline containing 0.5% formalin. Marked capsular swelling could be demonstrated in organisms thus treated. This suspension was used for intravenous injection of rabbits in 3 series of 6 daily inoculations each, with 8-day rest periods intervening. The total quantities of vaccine used in the successive series were 0.5, 0.85, and 1.85 cc respectively. Following the first 6 weeks' schedule as outlined above, the second and third series doses were alternated for 3 months. Subsequently, the third series doses were used every other week during the entire course of injection.

The rather crude test of potency was a determination of the highest dilution of the serum that produced capsular swelling of the organisms. The average titers after 6, 8, 15, 20, and 24 weeks were respectively 1:10, 1:20, 1:40, 1:80, and 1:320. Two determinations were made on each period-sample.

The antiserum produced by this method has yielded encouraging results in the treatment of children with *H. influenzae* meningitis. Untreated serum was used intrathecally, a globulin-fraction intravenously.