

## A NOTE ON THE PRODUCTION OF ANTIPNEUMOCOCCUS SERA.

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The value of homologous antipneumococcus serum as a therapeutic agent in lobar pneumonia caused by Type I pneumococci, has been demonstrated at The Rockefeller Institute Hospital during the past 3 years. It is desirable that the serum should be brought within reach of a larger section of the community than has been possible hitherto, but difficulties in production and administration of the serum have made this impossible. The present report deals entirely with the production of antipneumococcus sera.

In August, 1915, immunization of horses to pneumococci of the Types I, II, and III was commenced at the farm of the New York State Department of Health near Albany, under unfavorable conditions owing to inadequate facilities at the laboratory and the farm, and to irregularities in the injections caused by bad and occasionally impassable roads and by exposure of cultures to intense cold during the winter months. None of the horses immunized was under 11 years old. It was therefore thought that if potent sera could be produced under these adverse conditions, the success would strengthen the belief in these methods of immunization, and suggest possibilities for their broader application.

For immunization the method of Cole<sup>1</sup> with washed culture sediment and the method of Wadsworth,<sup>2</sup> using whole fresh 18 hour cultures, were followed. Beginning with weekly injections, or injections on 3 successive days each week, of heated cells or cultures, these were later followed by increasing doses of living organisms.

<sup>1</sup> Cole, R., *J. Exp. Med.*, 1912, xvi, 644.

<sup>2</sup> Wadsworth, A. B., *J. Exp. Med.*, 1912, xvi, 54, 78.

The potency of the sera was tested by protection tests on mice and by agglutination reactions. Intraperitoneal injection of control mice with 0.000001 cc. of the standard strains of Type I, II, or III, received from The Rockefeller Institute Hospital, and used for immunization and for these tests, was invariably fatal in less than 40 hours. Of three horses immunized against Type I organisms, 0.1 cc. of serum from No. 26 (culture sediment) or No. 28 (whole culture) protected, as a rule, against 0.4 cc., and not infrequently against 0.5 cc. of homologous culture. In three tests where 0.6 cc. of culture was used, the mice did not survive. 0.1 cc. of serum from No. 27 (culture sediment) protected, as a rule, against 0.2 cc., and sometimes against 0.3 cc. of homologous culture.<sup>3</sup> Wide variations similar to those reported by other workers were shown in the protection tests with Type II sera. 0.1 cc. of serum from No. 16 (culture sediment) or from No. 32 (whole culture) protected at times against 0.1 and 0.01 cc. of culture, and quite constantly against 0.001 cc.; a slightly lower protective titer was given by serum from Horse 29 (culture sediment).

Agglutination reactions indicated an inverse relation between the agglutinating power and the protective value of the sera, as in each type the horses with a lower protective index gave serum having a higher agglutination titer. Owing to the wide range of variations in agglutination reactions, due to differences in the density of the pneumococcus suspensions, satisfactory comparison with previous agglutination tests made by other workers is practically impossible.

While successful active immunization of rabbits, sheep, and goats with Type III organisms (*Pneumococcus mucosus*) has been reported, the production of sera of sufficient potency to agglutinate fresh untreated homologous organisms or to confer passive immunity on mice has apparently been unsuccessful hitherto. These failures have been considered to be due to the large mucoid capsule and tenacious slimy material produced by and surrounding the organ-

<sup>3</sup> The serum from Horse 27 has since protected 0.1 cc. against 0.4 cc. of culture. Experimental tests of different methods for standardizing the potency of antipneumococcus sera are in progress. Meanwhile in our experience more satisfactory results are obtained when 0.1 cc. of Type I serum instead of 0.2 cc. is used in protection tests on mice.

isms, which necessitated preliminary treatment, such as with a weak acid, according to the method of Porges,<sup>4</sup> before agglutinations could be obtained in the homologous sera.

For purposes of study and comparison, the immunization of a horse to Type III organisms (culture sediment) was carried on in conjunction with the horses of Types I and II.<sup>5</sup> The strain used for immunization while at first less virulent soon reached that of the other standard types, 0.000001 cc. killing a mouse within 40 hours. Protection tests showed that the serum possessed a definite though slight and variable protective action for mice. 0.2 cc. of serum in one instance protected against 0.001 cc. of culture, while in other tests mice receiving the same amounts of serum and culture died in 35, 63, or 70 hours. The same tests with several other Type III strains gave similar results.

In agglutination tests with fresh untreated Type III organisms, in concentrations of 1:1, agglutination was prompt and striking, usually showing before the tubes were placed in the water bath. Within 15 to 30 minutes agglutination was generally complete, and a loose cap had formed similar to those often seen in tests with Type I and Type II organisms and their homologous cultures. While agglutination in concentrations of 1:1 and 1:10 occurred as promptly as with Type I and Type II in their homologous sera, it was entirely absent in dilutions above 1:40. Undiluted serum obtained 5½ months after the last immunizing injection agglutinated Type III organisms with equal promptness; serum of 1 month later, however, caused no reaction. This is of interest because the abscess which had been discharging during the previous six months had healed meanwhile. All strains of the *mucosus* type tested, which were morphologically and culturally typical, agglutinated promptly with

<sup>4</sup> Porges, O., *Wien. klin. Woch.*, 1905, xviii, 691. von Eisler, M., and Porges, O., *Centr. Bakteriolog., 1te Abt., Orig.*, 1906, xlii, 660. Hanes, F. M., *J. Exp. Med.*, 1914, xix, 38.

<sup>5</sup> During the course of immunization a large abscess formed at the site of injection from which Type III organisms were isolated. Later a second abscess of a similar nature developed between the shoulder blades. It would be of interest to know the connection, if any, between these local foci of infection and the exceptional potency which the serum later developed.

one exception, but several strains which did not dissolve in bile failed to agglutinate. In a number of instances Type III organisms, which might readily have been classified as Type IV, owing to apparent absence in inoculated mice of the characteristic sticky peritoneal exudate and to the unusually small capsule of the organisms, were agglutinated promptly in Type III serum. Conversely, absence of agglutination in Type III serum made possible prompt exclusion from the Type III group of pneumococci obtained from the peritoneum of mice, in which a slightly viscous exudate was apparently present. The serum is, therefore, of distinct value in the diagnosis of pneumococcus types and should be used as a routine procedure together with the sera of Types I and II.

#### SUMMARY.

Horses immunized to Type I pneumococci developed serum, 0.1 cc. of which protected against 0.5 cc. of a virulent culture, 0.000001 cc. of which killed mice in less than 40 hours. Protective tests of serum from horses immunized to Type II organisms varied, 0.1 cc. protecting, however, in certain instances against 0.1 and 0.01 cc. of virulent homologous culture. Types I and II sera obtained in our experiments with culture sediment and whole culture did not vary markedly for a given type and corresponded closely in their protective titer with samples of sera received from The Rockefeller Institute Hospital. It is therefore evident that the following minimum standard of 0.1 cc. of serum to protect mice against at least 0.2 cc. of virulent cultures can and should be maintained when serum is to be used for the treatment of cases. By further study and comparison of these different methods of immunization it is hoped that sera of greater potency may be produced, but as yet this has only been accomplished in exceptional instances.

A horse immunized with Type III (*Pneumococcus mucosus*) developed serum having a slight degree of protection for mice against the corresponding organisms. This serum was sufficiently potent, however, to cause prompt and complete agglutination when combined with fresh untreated homologous organisms, thus avoiding the preliminary treatment to remove the capsule which has previously been held necessary. As a diagnostic aid in the differentiation of pneumococcus strains, the serum has proved of distinct value.