

phate is added to make the dialysate one-twentieth normal (50 cc. normal sodium chloride or sulphate to each liter) and placed in a cold room over night to lower the temperature to about 5°C. Two-tenths percent trikresol is then added and the mixture adjusted to pH 5 to 5.1, using gamma dinitrophenol 0.025% solution as indicator. The antibodies are soluble at this pH and salt content. This mixture is allowed to remain at about 8°C. for 4 hours, during this time the insoluble substance fibrin, fibrinogen, euglobulin and the chill producing substance will have flocculated and settled to about 1/20 of the fluid volume. At this cold temperature it is then filtered clear through paper pulp (filtration is very rapid, 20 liters within a half hour). The filtrate is adjusted to pH 6.8 using para nitrophenol 0.1% solution as indicator, and allowed to stand over night at cool temperature for further precipitation, principally fibrin like substance at this pH. It is then filtered through paper pulp and trial tubes set up to determine the amount of distilled water necessary to precipitate the antibodies. This rarely requires more than two and a half volumes of distilled water. The required water is added and the precipitate allowed to settle in cool room over night. The following morning the supernatant fluid is decanted and the remainder of the precipitate centrifuged. The centrifuged precipitate is roughly estimated in cubic centimeters and a like amount of one percent sodium chloride containing 0.8% phenol is added and the precipitate dissolved. The volume measured and diluted with 0.5% salt solution containing 0.4% phenol to one-tenth of the original plasma used. The solution is kept in a cool place for at least 24 hours for any clouding or precipitation that may occur. It is filtered through paper pulp to clarify and 1% sodium chloride added to bring the salt content up to 1.5%. It is then filtered (Berkefeld), tested for sterility and potency.

5270

A Modified Method for the Production of Antipneumococcus Serum in Horses.

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The method of preparing antisera consisting of the use of formalinized sediment of 18-hour pneumococcus broth culture injected

intravenously into horses is one in general use. The comparison of the therapeutic value of such antisera on the basis of their relative mouse unit content has been generally adopted.

A previous communication by one of us¹ demonstrated the possibility of producing a potent antiserum by means of the injection of pneumococcus pleural exudate intravenously. This was based on the hypothesis that such an antigen was likely to contain products from both the invading organism and the reacting tissue of the host, and would therefore be more nearly comparable to that produced under natural conditions during the disease itself.

Knowing that animals under immunization respond with a greater concentration of antisubstances when soluble antigens are injected intramuscularly than when administered intravenously, it was decided to immunize horses by means of phenolized pneumococcus pleural exudate intramuscularly and formalinized sediment of 18 hour broth culture intravenously.

The sera of horses thus immunized have been tested by the mouse protection test, and fall in 2 groups, those of high unit value and those of low unit value. When such sera were compared by the method suggested by Goodner² with control antipneumococcus sera of high and low titre prepared by vaccine administered intravenously, it was found that their therapeutic value was disproportionate to their mouse unit content indicating the possible presence of added therapeutic substances resulting from the modified mode of immunization.

5271

The Titration of Pneumococcus "Exudate" Antisera.

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In a previous communication¹ a method for the production of anti-pneumococcus serum by means of the immunization of horses with type specific pneumococcus pleural exudates was reported. Sera pre-

¹ Curphey, T. J., and Baruch, M. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1929, **26**, 687.

² Goodner, K., *J. Exp. Med.*, 1928, **48**, 1.

¹ Curphey, T. J., and Baruch, H. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1929, **26**, 687.