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 us[1] 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 Goodner[2] 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.

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The Titration of Pneumococcus "Exudate" Antisera.

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In a previous communication[1] a method for the production of antipneumococcus 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.

pared by this method were found to contain protective substances in about the same concentration as other sera prepared by intravenous immunization with pneumococcus vaccine, when tested in mice. However, in view of the fact that it was felt that the "exudate" antisera might contain certain anti-substances resulting from a possible antigenic effect of the cellular components of the inflammatory exudate (acting either alone, or in the nature of a haptene to the type specific pneumococcus protein radicle therein present) it seemed necessary to titrate such antisera under conditions in which both the bacterial and the cellular antigenic components would co-exist and be capable of some quantitative measurement. The method of producing an acute inflammatory lesion by intradermal injection of the pneumococcus as outlined by Zinsser[2] and later studied in detail by Goodner[3, 4] provided at the local site of inflammation both the bacterial and the cellular components in the course of an active infection of the skin, and therefore seemed suitable for measuring the potency of such "exudate" antisera.

Tests conducted with serum from a horse immunized with Type I pneumococcus exudate intravenously, and controlled with several sera from horses immunized with vaccine show that a larger number of rabbits are protected against a standard dose of Type I pneumococcus culture injected intradermally when they receive a given mouse unit dose of "exudate" antiserum, than when a similar dose of "vaccine" antiserum is administered at the same time after infection. Thus, of a group of 53 rabbits receiving a skin infection of about 200,000 organisms each, of 27 treated with 450 units of "exudate" antiserum, six hours after infection, 19 survived (70.4%) while of a similar group of 26 receiving 450 units of "vaccine" antiserum six hours after infection, only 9 (34.7%) survived.

These results while they represent the titration of a single "exudate" antiserum, seem striking enough to warrant the assumption that such an antiserum contains substances other than antibacterial antibodies, seeing that control antibacterial sera were far less able to affect the course of the local inflammatory lesion and to lead to as high a survival rate. Furthermore, work at present in progress with other samples of "exudate" antisera indicate that the difference observed above is not that of individual horse variation, lending support to the hypothesis that the cellular component of the "exudate"

[2] Zinsser, H., *Boston Med. and Surg. J.*, 1925, **192**, 1191.

[3] Goodner, K., *J. Exp. Med.*, 1928, **48**, 1.

[4] Goodner, K., *J. Exp. Med.*, 1928, **48**, 413.

antigen is an important contributory factor in the production of pneumococcus antisera.*

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A Simplified Method for Quantitative Tissue Culture in vitro.

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Objects of Method: (1) to be able to observe the growth of tissue using high power microscopic objectives, as in the original cover slip method of Maximow; (2) to secure quantitative growth as has been made possible to a certain extent by the Carrel flask;* and (3) at the same time to retain the advantages of the cleverly devised Borel flask in which the bottom is detachable, so that the tissue can be fixed and stained without distortion, and thus retained as a permanent record of the tissue grown. The present method is a simplified adaptation and combination of all 3 of these techniques.

The simple apparatus consists of but 3 parts: (1) A glass ring 3 inches in diameter, 5-6 mm. in height, with parallel, flat, ground surfaces, 2-3 mm. in thickness. These are made from heavy pyrex tubing. (2) Two thin sheets of mica 3¼ inches in diameter. (3) An ordinary 4 inch petri dish.

Method: Absolute cleanliness of the glassware is essential We sterilize the glass ring within a petri dish, and sterilize the mica sheets separately in another petri dish for convenience in handling them. Working preferably in a sterile bacteriologic transfer room, to one edge of the sterilized glass ring is applied a rim of vaseline (1% paraffin) by means of a sterile wide mouthed pipette. One of the sterile mica sheets is placed upon it and pressed down. This is then turned upside down within the petri dish where it forms a chamber, the cover being the bottom, and the ring its wall. This is now ready to receive any medium, solid or liquid, and the tissue for culture. As the entire chamber is readily accessible by simply removing the cover of the petri dish, the fragments of tissue can be arranged

* We beg to express our gratitude to Dr. Wm. H. Park for his interest in this work and to Miss Lillian Gross for her technical assistance.

* Since presenting this paper we have learned that a somewhat similar, but more elaborate apparatus with a metal ring has been used by Carrel.