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# Blood clot method of immunization with observations on pneumococcus toxemia.

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Recent work in scarlet fever and the staphylococcus toxin work of J. T. Parker[1] have obviously suggested renewed inquiry into pneumococcus infections by similar methods. One of us discussed this in a paper of last June.[2] The thought has surely come to many bacteriologists. Moreover, it has been tried many times before; by the Klemperers,[3] by Mosny,[4] by Tizzoni and Panichi,[5] and, particularly, by Wadsworth,[6] who discussed the differences between the antitoxic effects of sera produced with culture filtrates and the antibacterial action of those produced with the whole bacteria. Thomas and Frederick Parker,[7] furthermore, pointed out microscopic necrosis in various organs not associated with the local presence of pneumococcus.

Savchenko's[8] papers of 1905 and the Dick studies show that a bacterial substance, poisonous for man and antigenic, may be relatively harmless for all the ordinary laboratory animals; and from J. T. Parker's work we learn that the toxicity and antigenic effectiveness of some poisons may be limited to individual tissues. We are therefore approaching the problem indirectly by immunizing horses with pneumococcus preparations in the hope that eventually the serum of these horses may display antitoxic potency rather than purely antibacterial effects in clinical test. In

1 Parker, J. T., *J. Exp. Med.*, 1924, xl, 761.

2 Zinsser, Shattuck Lecture, *Boston Med. and Surg. J.*, 1925, cxcii,1191.

3 Klemperers, *Berl. Kl. Woch.*, 1891, xxviii, 833 and 869.

4 Mosny, cited from Wadsworth loc. cit., *Arch. di med. Exp.*, etc., 1892, iv, 195.

5 Tizzoni and Panichi, *Centralbl. f. Bakt.*, Ref., 1905, xxxvi, 25.

6 Wadsworth, *J. Exp. Med.*, 1912, xvi, 54 and 78.

7 Thomas and Frederick Parker, Jr., *Arch. Int. Med.*, 1920, xxvi, 125 and 132.

8 Savchenko, *Russki Vratch.*, 1905, xxv, 797; cited from Park, *J. Am. Med. Assn.*, 1925, lxxxv, 1180.

the course of this work we have developed a method which we think may be useful not only for our purposes, but for the production of sera in the other diseases mentioned. We are publishing the method now, since we think it sufficiently promising to make general trial desirable. Incidentally, we believe it has furnished us further evidence that a toxic factor develops when pneumococci grow in the body under conditions analogous to those prevailing in pneumonia. Whether or not this toxic factor is antigenic remains to be seen.

We began by treating two horses with increasing amounts of 5 day blood broth culture filtrates of Type I pneumococcus. Resulting local reactions were rarely more than slight, and doses of 500 cc. could be given within 6 weeks after beginning treatment, occasionally producing slight temperature and transient local edema. In one horse, such filtrates were alternated with a modified Dochez method consisting in the injection of 20 to 30 cc. of agar inoculated in the syringe with virulent pneumococci. The agar injections resulted in violent local and sometimes systemic reactions, with eventual abscesses. In the other horse, filtrate injections were alternated with increasing intracutaneous doses of virulent pneumococci. After three months, as much as 50 cc. blood broth cultures of the organisms were so administered with astonishingly mild reactions. This mildness is of importance in its contrast with the severe results of the agar injections, and the very severe ones obtained by the new method. It is carried out as follows:

Normal horse blood is taken into potassium oxalate solution to prevent clotting. It is inoculated with pneumococci and incubated for about 24 hours. At this time a sufficient amount of calcium chloride is added and the mixture subcutaneously injected into the horse. A subcutaneous hematoma results in which the organisms continue to multiply. In early injections the oxalated blood is not incubated with pneumococci but inoculated just before injection. In such cases, not only calcium chloride but a little horse serum, to supply thrombin, is added. Grinnell has found that incubation of oxalated horse blood with pneumococci hastens coagulation, an observation which is being further studied by him.

The injection of 40 cc. of such an infected clot in the horse which had previously shown slight reactions only to intracuta-

neous doses of 50 cc. of virulent culture resulted in a violent local reaction with an edematous swelling, spreading, hot and tender, rise of temperature to 39.4, depression and failure to feed, this subsiding gradually in about 5 days. It is also interesting that the other horse, which had had previous agar treatments, reacted less severely to a similar injection, which we hope may indicate a developing immunity.

In order to determine in a preliminary way what it is that is probably going on in the injected blood clot, pneumococci were cultivated *in vitro* in human blood clots and with suitable controls of uninoculated blood clot extracts; a number of individuals were intracutaneously tested.

Susceptibility to these materials varies very much in individuals, which is encouraging in rendering less probable a non-specific toxicity attributable to cleavage of the protein. In susceptible individuals, such as one of the writers, 1/20 of a 1:10 dilution, representing in final calculation 1/400 cc. of the original culture material, gave rise to a painful, red and swollen area, reaching its maximum in about 36 hours, at that time having a red areola over an inch in diameter, hot and tender, with some pain up and down the arm; not completely fading for 2 weeks. Another individual developed a somewhat smaller but similar reaction with the same amount, and with a negative control, while others, with half this amount, namely, 1/800 cc. of the original material, had, in recent tests, three of them definite but slight reactions with negative controls, while one individual in this group was absolutely insusceptible.

We make no particular claims at the present time either as to the specificity of this poison or its antigenic properties. We believe, however, that it represents the substance which gives a severe reaction in the horses inoculated by the blood clot method, and that its potency, relatively low as it is as compared with other bacterial poisons, is still sufficient in susceptible individuals to more than account for the clinically observed toxemia in pneumonia. Our primary purpose in presenting these facts is to submit a method which, in its ease of performance and failure to cause abscesses, the production of localized bacterial foci without foreign substances and complete eventual absorption, should be freely available for the experimental study of serum production.

The observations herein reported were made possible by the

cooperation and, indeed, collaboration of our associate, Dr. Benjamin White, who is continuing to take an active part in the further studies of the pneumococcus serum and is beginning to apply the method to scarlet fever serum production. In collaboration with Dr. White, we are further attempting the effects of using this method with some other bacteria.

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### Effect of menotoxin injections on behavior of rats in the maze.

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Macht and Lubin[1] have described their studies on menotoxin first in these Proceedings and later in a fuller communication elsewhere.[2] It was shown that in the blood, sweat, saliva, milk and other secretions of menstruating women there is present a toxin, which is especially deleterious to plant protoplasm, but is also to a lesser degree toxic for animals and animal tissues. The chemical nature of this toxin was found by them to bear a relationship to oxycholesterin and allied bodies such as cholic acid.

It is well known that at the time of catamenia, the female organism undergoes profound metabolic and other physiological changes. Very common concomitants of menstruation are pain, malaise, nervous irritability, and psychic disturbances. In the present investigation an inquiry was made as to whether such symptoms may not be referred to the presence of menstrual toxins. Albino rats were trained to run in the circular maze, so as to perform that exercise in the shortest period of time and without errors. The rats were then injected with normal human blood serum on the one hand, and with blood serum from menstruating women, on the other hand, and the effect of the injections was observed. In order to avoid anaphylactic phenomena

[1] Macht, D. I., and Lubin, D., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 265.

[2] Macht, D. I., and Lubin, D., *J. Pharm. and Exp. Therap.*, 1924, xxii, 413.