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Intraperitoneal Injections of Pneumococcus in Animals Primarily Receiving Leucocytic Stimulating Antigens.*

HERBERT J. SCHATTENBERG AND WILLIAM H. HARRIS.

From the Department of Pathology, College of Medicine, Tulane University.

In a previous communication[1] we reported the difference in leucocytic response to the injection in animals of various antigenic substances. The differences were of a marked character, not only for the different antigens used but also for the route of injection chosen. Killed cultures of *B. typhosus* and *Staph. aureus* injected intraperitoneally resulted in the formation of the highest leucocytic increase. The peak of the rise appeared in from 6 to 8 hours. We also called attention to the phenomenon of local leucocytosis as a possible misleading factor in making such observations.[2] Some allusion to this feature had previously been made by Garrey and Butler.[3, 4]

Steinberg[5] reports that 4 intraperitoneal injections of heat killed *B. coli* at daily intervals, protects 65% of animals against an artificially produced peritonitis caused by a living *B. coli* suspension administered on the 5th day. Gay and Clapole[6] have shown that the "carrier state" in rabbits can be prevented if previously immunized rabbits are injected with a live suspension of *B. typhosus*. Their experiments show that there is a much greater leucocytic response when immunized rabbits are injected than when normal rabbits are used and in the latter case the "carrier state" cannot be prevented.

To ascertain the protective influence of induced leucocytosis, 47 white mice received intraperitoneal injections of the following antigenic substances for the purpose of producing leucocytic reactions in approximately 6 hours as controlled by the previously reported experiments:[7] (1) Suspension of killed *B. typhosus*. (2) Suspension of killed *Staphylococcus aureus*. (3) Detoxicated suspension of killed pneumococcus. (4) Sterile milk.

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[1] Harris and Schattenberg, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 265.

[2] Schattenberg and Harris, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 269.

[3] Garrey, W. E., and Butler, Virginia, *Am. J. Physiol.*, 1929, **90**.

[4] Garrey, W. E., and Butler, Virginia, Reprinted from *Proceedings of the Staff Meetings of the Mayo Clinic*, 1929, **4**.

[5] Steinberg, Bernhard, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 16.

[6] Gay, F. P., and Claypole, E. J., *Arch. Int. Med.*, 1914, **14**, 662.

[7] Harris and Schattenberg, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 265.

After 6 to 8 hours these animals were injected intraperitoneally with lethal doses of Pneumococci types I, II, and III. These cultures were grown in Avery's medium and suspended in saline solution to produce a suspension containing approximately 300,000,000 organisms per cc. The amounts given, varying from 0.1 to 0.25 cc., were adjusted so that death of the animals could be controlled and occurred at periods varying from 3 to 24 hours.

The animals in the various series received a respective antigenic injection approximately 6 hours previous to the administration of the infecting dose of the pneumococcus. In one series, 2 antigenic doses were given at 24 hour intervals and an infecting dose 6 hours following the last injection. Control animals for the infecting dose were run with each series and the effects of the antigenic injection were also noted in other control animals.

While in certain instances some of the supposedly protected animals survived for several hours longer than the infection control animal, at other times the control lived somewhat longer than those that had received the antigenic dose or doses.

Our procedure does not conform to that used by Steinberg in that our infecting microorganism was more virulent and that repeated injections to produce a hyperleucocytic immunity were not administered.

The results of our experiments would indicate that in white mice, the injections of various antigenic substances employed as stimulators for a leucocytic increase, failed to show any evidence of protection to this animal against the production of fatal pneumococcal peritonitis.

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Effects of Toxic and Non-toxic Doses of Thorium Dioxide in Various Animals.

C. J. TRIPOLI AND E. V. HAAM.

From the Departments of Pathology of Charity Hospital and Louisiana State University Medical Center.

The value of the method of roentgenographic visualization of the liver and spleen in the human is, in our opinion, dependent only upon the possible dangers involved in the intravenous administration of stabilized colloidal suspensions of thorium dioxide (Thorotrast).