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Preparation of an Active Undenatured Antigen from Hemophilus Pertussis.*

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Any study of the use of vaccines in whooping cough must take into consideration two basic facts, first that the causal agent is generally conceded to be the Bordet-Gengou bacillus,[1] and second, that this organism is capable of yielding high antibody titres in the vaccine treated host, as well as in the convalescent patient.[2] On purely *a priori* grounds then, one would rather expect to find formidable statistics evidencing the protective value and perhaps the therapeutic efficiency of pertussis vaccine. Yet the past 25 years has witnessed no convincing trend in this direction. That the outlook is not altogether dismal is indicated by such clinical series as those of Sauer,[3] and Madsen,[4] and it seems likely that much of the discordance in the literature reflects the multiplicity of methods used in preparation of the vaccine rather than a failure of the basic tenets of vaccine therapy.

Krueger[5] has recently emphasized the rôle of denaturation reactions in altering the antigenicity of bacterial preparations and has pointed out that these reactions probably occur to a very considerable degree during the physical and chemical manipulations incident to the killing of bacterial suspensions, as ordinarily carried out. To obviate the denaturation factor somewhat he has physically disrupted living bacterial cells of various kinds and has put into solution or suspension the cellular components released by this method. After removal of residual live cells by ultrafiltration the filtrate is employed as an antigen. The present paper details the procedure followed in making *Hemophilus pertussis* antigen of this type for clinical experimental studies in immunization and therapy.

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1 Gardner and Leslie, *Lancet*, 1932, **1**, 9.

2 Chievitz and Meyer, *Ann. de l'Inst. Past.*, 1916, **30**, 503.

3 Sauer, L., *J. Am. Med. Assn.*, 1933, **100**, 239.

4 Madsen, T., *Deut. Med. Wchnschr.*, 1929, **55**, 559.

5 Krueger, A. P., *J. Inf. Dis.*, in press.

We were convinced by preliminary work that the present antigen should be prepared from smooth pathogenic types (Phase I of Leslie and Gardner)[6] and to that end constantly renew our stock strains, replacing the latter at brief intervals with new strains obtained from cough plates. The organisms are grown in Roux flasks on potato-extract-glycerine-agar medium enriched with 25% of human blood. Human blood is employed instead of horse blood because we desire to rule out the possibility of sensitizing our patients to foreign serum proteins adsorbed on the bacteria, and because human blood appears to maintain the active antigenic phase of *Hemophilus pertussis* more consistently.

After 48 hours' incubation at 37°C. the growth is harvested in sterile Locke's solution (pH 7.0). The suspension is given one preliminary washing in Locke's solution and is then passed through filter paper in a Gooch filter to eliminate agar particles. The bacteria are then alternately centrifuged and resuspended in fresh Locke's solution for 3 successive washings, discarding the supernatant each time. The last supernatant is always clear, and from the sediment a final suspension is made up so that each ml. contains about 5×10^{10} bacteria as determined by the centrifuged sediment method.[7] The heavy bacterial suspension is then transferred to a special type of ball mill[8] where it undergoes grinding for 12 hours. The ground-up suspension is filtered through a 4.5% acetic collodion membrane,[9] this final step yielding a water-clear filtrate which constitutes the antigen. To it may be added 1:1,000,000 merthiolate as a bacteriostatic without causing detectable denaturation.

The antigen regularly contains approximately 4 mg. of nitrogen per 100 ml. of which 90% is protein nitrogen. The preparation gives a positive Biuret test, a positive Molish test, a positive xanthroproteic test, a negative Hopkins-Cole reaction, a positive Adamkiewicz test, and a negative Millon test.

It is distinctly non-toxic, as evidenced by our failure to elicit temporary or cumulate toxic effects in rabbits and guinea pigs by daily subcutaneous, intradermal and intravenous injections. Intradermal injections of antigen into rabbits followed within 20 hours by intravenous injection of the same material failed to produce local tissue reactions such as those reported by Mishulow,

[6] Leslie and Gardner, *J. Hyg.*, 1931, **31**, 423.

[7] Krueger, A. P., *J. Gen. Physiol.*, 1930, **13**, 553.

[8] Krueger, A. P., *J. Inf. Dis.*, in press.

[9] Krueger, A. P., and Ritter, R. C., *J. Gen. Physiol.*, 1930, **13**, 409.

Mowry and Scott[10] who used toxic filtrates of *H. pertussis*. If, however, the final intravenous injection was performed with a live suspension of the organism after preliminary skin sensitization with the present antigen, a typical local erythematous response, later progressing to skin necrosis, was evinced.

Rabbits immunized by combined intradermal and subcutaneous injection of the antigen were capable of withstanding intravenous injection of $2x10^{10}$ virulent organisms—an amount which kills control animals of like weight within 20 hours. It did not seem likely that further protection experiments with animals not naturally susceptible to clinical whooping cough would yield information of any value. Therefore, after adequate experiments on the clinical and laboratory staff to demonstrate its harmlessness to humans, the antigen was subjected to clinical trial.

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Effect of Desiccated Beef Testis Upon Sex Glands of the Rat.

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The prostate gland of the rat shows a marked increase in weight in aged males.[1] McGee[2] and Gallagher and Koch[3] have shown that the testicular hormone of the beef testis is lipoid soluble. Moore and McGee[4] state that the lipoid fraction of the beef testis possesses the same activity as the internal secretion of the testis, judged by its effect on spermatozoa in isolated epididymes.

We have fed beef testis to mature male rats in an attempt to determine whether it might have any effect on the increase in weight of the prostate gland, as it tends to occur in the aging animals. The testes were ground and desiccated at 65°C., without removal of fat or extractives. The desiccated testis was then refrigerated and fed in 0.2 gm. amounts, 5 times a week.

The experimental animals gave no visible evidence of departure

[10] Mishulow, Mowry and Scott, *J. Immunol.*, 1930, **19**, 227.

[1] Donaldson, H. H., The Rat. Wistar Institute, Philadelphia. 1924.

[2] McGee, L. C., *Proc. Inst. Med. Chicago*, 1927, **6**, 242.

[3] Gallagher, T. F., and Koch, F. C., *J. Biol. Chem.*, 1929, **84**, 495.

[4] Moore, C. R., and McGee, L. C., *Am. J. Phys.*, 1928, **87**, 436.