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# A Proposed Mouse Protection Unit for Anti-Meningococcus Serum.

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The use of mucin for the enhancement of the infectivity of meningococci in the mouse has now been studied for over 5 years.[1] The first use of this technic in the titration of the protective action of sera was reported over 3 years ago.[2] Subsequently, other laboratories have reported the titration of protective antibodies in antimeningococcus serum and in each case the details of technic employed have varied slightly or markedly.[3-9] In order that values obtained in the titration of antimeningococcus sera in various laboratories may be comparable, that figures obtained by the use of the test may have a wide application, and that sera used for therapeusis may be studied on a basis which allows quantitative comparison, it has seemed advisable to set up and distribute a tentative standard polyvalent serum as a control and to assign to this serum certain values so that other sera may be referred to it quantitatively. It has also seemed desirable that a uniform technic for performing the mouse protection test be used.

In order to obtain a polyvalent serum and data for this purpose, a serum (of which ample amounts were available) was selected because it resembled closely the older control serum M 18 in its protective capacity and other immunological reactions. This has been designated M 19. Repeated estimations of antibody nitrogen for Group I* were carried out with this serum and it was determined that the

[1] Miller, C. P., *Science*, 1933, **78**, 340.

[2] Rake, G., *J. Exp. Med.*, 1935, **61**, 545.

[3] Miller, C. P., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1140.

[4] Rake, G., *Ibid.*, 1935, **32**, 1175.

[5] Cohen, S., *J. Immunol.*, 1936, **30**, 203.

[6] Mishulow, L., Melman, M., and Sklarsky, R., *J. Lab. and Clin. Med.*, 1936, **21**, 406.

[7] Miller, C. P., and Castles, R., *J. Inf. Dis.*, 1936, **58**, 263.

[8] Rake, G., *Can. Pub. Health J.*, 1937, **28**, 265.

[9] Pittman, M., Branham, S. E., and Sockrider, E. M., *U. S. Pub. Health Rep.*, 1938, **53**, 1400.

* By Group I is meant only those strains known as Type I and Type III; by Group II only those strains known as Type II.

serum contained 0.65 mg of antispecific polysaccharide nitrogen[10] per cubic centimeter. It has been shown[8] that the Group I protective power of antimeningococcus serum probably parallels the type-specific antibody nitrogen content. This has also been found to be the case with antipneumococcus sera. In the latter case 1.00 mg of antibody nitrogen is found to be equivalent to approximately 1000 protective units, a convenient figure. In view of these facts, it seemed logical to give a tentative value of 650 protective units per cubic centimeter for the Group I titer of serum M 19, and this figure has been tentatively adopted.

The present lack of sufficiently pure Group II specific substance has precluded the use of antibody nitrogen estimation in arriving at a figure for the Group II protective antibodies. In common with all other polyvalent antimeningococcus sera so far investigated, the Group II titer for M 19 is far lower than the Group I. On the basis of the protective capacity of the serum against Group II strains as compared to the like capacity against Group I, a figure of 25 protective units per cc would seem to be approximately correct and it is suggested that this figure be tentatively adopted for comparative purposes. This tentative protective unit for both Groups I and II is for purposes of research, and is not to be considered as an official standard unit for antimeningococcus serum.

For the mouse protection test the cultures used, whether of Group I or Group II, should be of maximum virulence. This means that 1 cc of a $10^{-9}$ dilution which contains on the average 2 organisms should kill over 50% of mice on intraperitoneal inoculation. Such strains are available. The virulence of these strains can usually be maintained by the preparation and use of cultures which have been dried from the frozen state or by weekly passage on serum dextrose agar. It can be more certainly maintained by passage through mice at least once a week and maintenance on serum dextrose agar or by daily passage on 4-5% blood agar.

In order that this high virulence may be rendered apparent and usable it is, of course, essential that mucin be used. Granular mucin (Wilson) is recommended, and suspensions are made according to the description of Miller and Castles.[7] †

To obtain consistent results with small numbers of mice it is advisable that a pure line inbred stock of proven susceptibility be used

10 Scherp, H. W., and Rake, G., *J. Exp. Med.*, 1936, **63**, 547.

† In the past different lots of the mucin preparation have varied considerably and this has introduced an element of difficulty. Recently, the Wilson Laboratories have supplied us with a new preparation which gives most satisfactory results and which, it is believed, can be reproduced at will.

at weights between 16 and 20 g. Such a stock is available in this country as the so-called "Swiss" mouse.

In carrying out the actual test, blood agar cultures 4 to 6 hours old are used. The culture is suspended in broth and standardized to give 2,000,000,000 organisms per cc by some method such as the Gates Turbidimeter or comparison with standard suspensions of silica. Dilutions from this suspension are made at $10^{-1}$ and $10^{-2}$ in broth and at $10^{-3}$ through $10^{-9}$ in mucin.

In the meanwhile, progressive twofold dilutions of the control, or standard, serum and of the unknown sera have been made in saline. Three dilutions are used, as a rule, but more may be employed as long as both control and unknown have the same number. The dilutions most suitable for the control serum will be known beforehand; those for the unknown can be determined roughly in preliminary tests. A minimum of 5 mice are inoculated with each of the 3 (or more) dilutions, which are so prepared that the required dose of serum will be contained in 0.5 cc. Serum inoculations are given intraperitoneally. An hour later, all mice receiving serum are given 1 cc of a $10^{-3}$ or $10^{-4}$ dilution of meningococci in mucin intraperitoneally. Luer-Lok syringes and 24 gauge needles are suggested for this purpose. A control of the culture virulence is carried out in every test by inoculating a minimum of 3 mice in each dilution with $10^{-8}$ and $10^{-9}$ suspensions of meningococci in mucin (20 and 2 organisms approximately).

The mice are observed for 96 hours after the inoculation and deaths recorded in the usual manner. Given a control serum of agreed quantitative value in protective units the titers of unknown sera are worked out by the use of the formulæ suggested by White[11] or by Reed and Muench.[12]

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[11] White, B., *The Biology of the Pneumococcus*, 1938, N. Y.

[12] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.