It appears, therefore, that the virus obtained from human influenza is a distinct entity and is etiologically related to the human disease.

8017 P

A Method for Titrating the Protective Action of Antimeningococcal Serum.

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Miller[1] has described a method for the production of experimental meningococcal infection in mice. It consisted in brief of the use of a 6% mucin suspension buffered at 7.4 as a medium in which the organisms were suspended prior to intraperitoneal inoculation. More recently Miller has modified the technique of preparing the mucin.[2] A 5% suspension is now prepared, it is sterilized in the autoclave at 10-15 lb. pressure for 15 minutes, sterile dextrose solution is then added to a final concentration of 1%, and the pH adjusted to pH 7.4 with sterile buffer solution.

Using such a mucin suspension, the intraperitoneal virulence of meningococcus strains can be titrated,[1, 3] and consistent results will be obtained when pure breeds of susceptible mice are employed. It has been found, in accord with Miller's work, that freshly isolated strains may kill when the cultures are diluted as far as 10^{-8}, that is approximately 20 organisms. A brief report has been made elsewhere[3] on the application of this experimental meningococcal infection to the test of sera for their protective activity. That report dealt chiefly with the content of protective antibodies in the serum of carriers of the meningococcus and in the serum of normal individuals. The high protective value of some antimeningococcal sera was demonstrated but no titration was carried out.

In testing the intraperitoneal virulence of strains, 14 to 18-hour cultures on 10% rabbit's blood pneumococcus agar plates are washed off with normal saline and the suspension is diluted in saline and adjusted with a Gates turbidometer to the standard of 2,000,000,000 organisms per cc. Serial dilutions 1:10 are made in mucin and

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

[2] Miller, C. P., personal communication.

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

range between 10^{-1} (200,000,000 organisms) and 10^{-8} (20 organisms). Inoculations are made intraperitoneally into selected susceptible strains of Rockefeller Institute mice, into Swiss and into white-faced breeds.[4, 5] Most mice are dead by the end of the 2nd day. Deaths from the specific infection are very rare after the 4th day.

The intraperitoneal virulence of a strain can be maintained by passage from mouse to mouse and may even increase ten or a hundredfold with such passage.

In testing a serum for protective antibodies, ½ cc. of the serum diluted 2:5 in normal saline is inoculated intraperitoneally half to one hour before the intraperitoneal inoculation of organisms is made. Thus, in one test 3 series of mice were tested with dilutions of a Type I culture in mucin from 10^{-1} to 10^{-5}. One series of mice was given a monotypical Type I antimeningococcal serum, another received normal human serum and the third received no serum at all. All mice receiving no serum or receiving normal serum died, whereas the Type I serum protected in every dilution, that is to say, protected against at least 100,000 minimal lethal doses.

In titrating the protective power of a serum, 2 methods have been adopted. The serum has been diluted out to 1:640 and ½ cc. inoculations of the different serum dilutions have been made one-half to one hour before infection; or the serum dilution and amount inoculated have been kept constant and the time at which the serum inoculation is given has been varied out to 11 hours after the infecting inoculation.

It has been found that the protective value of the serum falls more or less regularly with serum dilution and with the time interval after inoculation. Thus, in a titration of a monotypical Type I antiserum against a virulent Type I strain there was no demonstrable difference between the protective value of serum dilutions 2:5 and 1:10, but a dilution of 1:160 gives significantly less protection and the dilution of 1:640 hardly protects at all. A serum dilution of 2:5 given 30 minutes beforehand is more efficacious in protecting the mice than when given 4 hours and 8 hours after infection, but serum given this late protects against 1,000 M.L.D.

Heterologous antimeningococcal sera give some protection but it is less than that of the homologous serum or polyvalent sera. Thus, Type II serum protects against about 100 minimal lethal doses of Type I organisms when given in a dilution of 2:5 30 minutes before

[4] Webster, L. T., *J. Exp. Med.*, 1933, **57**, 793.
[5] Webster, L. T., *J. Exp. Med.*, 1933, **57**, 819.

TABLE I.

					Type I						Polyvalent X					
					2:5 30 min. before		1:160 30 min. before		2:5 10 hrs. after		2:5 30 min. before		1:160 30 min. before		2:5 10 hrs. after	
	No serum		Normal serum													
10^{-2}	<23	<23	<23	<23	<23	<23	<23	<23	<10	<10	S	S	<23	<23	<10	<23
10^{-3}	<23	23	<23	<23	S	S	26½	<71	<23	<23	S	S	<23	S	<23	25½
10^{-4}	<23	23	<23	24	S	S	<23	<28	11½	24½	S	S	<23	26½	<10	10½
10^{-5}	<23	<23	<23	23	S	S	S	S	23	S	S	S	S	S	<47	S
10^{-6}	<23	25	25½	31	S	S	S	S	S	S	S	S	S	S	S	S
10^{-7}	<47	<71	28	S	S	S	S	S	S	S	S	S	S	S	S	S

1 cc. of each culture dilution given intraperitoneally to each mouse—two in each dilution. ½ cc. of serum given. Dilution of serum and time of inoculation indicated. Time of death in hours indicated. < = mouse died during night in less than the number of hours indicated. S = survival for 5 days.

infection, but fails to protect in a dilution of 1:160 30 minutes beforehand or in a dilution of 2:5 10 hours after infection.

This protection test is being used in the comparison of certain of the polyvalent sera now on the market. All of those tested show good protection (namely against 100,000 m.l.d.) when given in a dilution of 2:5 one-half to one hour before infection. On titrating them, they have been compared with the monotypical Type I antimeningococcal serum prepared in this laboratory. Table I shows such a comparative test. It will be noticed that the normal human serum does not protect. The polyvalent serum gives as good or slightly better protection than does the homologous serum. Both antimeningococcal sera protect when diluted 1:160 (against 1,000 m.l.d.) and when given in a dilution of 2:5 10 hours after infection (against more than 100 m.l.d.).

Thus, the use of Miller's technique has allowed one to titrate the virulence of freshly isolated meningococcus strains and to develop a protection test for titrating antimeningococcal serum. The results in both instances are consistent only when pure breeds of susceptible mice are used. In our hands the use of unselected stock mice has led to results which, on account of their inconsistency, are highly unsatisfactory.

8018 P

Rate of Lymph Flow in Edematous Skin of Cardiac and Renal Disease.

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In normal skin an intradermal injection of deeply colored vital dye renders the lymphatics visible.[1] In a few minutes some of the dye drains away into the deeper channels appearing like colored streamers when seen through the skin. Scores of tests on normal volunteers have shown these colored streamers to be long or short under conditions known to increase or decrease lymph flow respectively. The method, to be described elsewhere, has been used to compare the rate of lymph flow in the edematous skin of cardiac and nephritic patients.

In more than 60 experiments upon 14 individuals with cardiac

[1] Hudack, S. S., and McMaster, P. D., *J. Exp. Med.*, 1933, **57**, 751.