that the neutral pH of all solutions is 7.00 at any temperature.[1,2] By definition this is the neutral point only of pure water at 24.9°C. At 35°C $K_w = 2.089 \times 10^{-14}$ [7] so that the neutral pH would be 6.84 for water. The change in pH of buffered solutions with temperature depends upon the buffers present and does not follow the same rules which define the neutral pH. For values near pH 7.0 it is, therefore, better to record the pH as found and the temperature at which the measurement was made without defining its relationship to the neutral point.

Summary. Glass electrodes were constructed for measuring the nasal pH of hamsters, ferrets, and rats *in situ*. The normal pH was found to be 7.2 at 35°C.

[7] Harned, H. S., and Owen, B. B., *Chem. Rev.*, 1939, **25**, 31.

14805

Type Specific Meningococcic Agglutinins in Human Serums. I. Description of Method.

CAROLYN R. FALK AND EMANUEL APPELBAUM. (Introduced by J. T. Freund.)

From the Bureau of Laboratories, Department of Health, City of New York.

The presence of meningococcic agglutinins in human serum has been investigated by a number of observers[1] whose studies showed a great variation regarding agglutinin titers and the possible significance of the presence of the antibodies. A difficulty in evaluating these investigations has been the varied technics used in carrying out the agglutination test.

Since April 1, 1942, we have studied serums from 685 individuals for the presence of Group I, Group II, and Group II-alpha agglutinins. These specimens were obtained from the following sources: 276 cases of clinically typical meningococcic infection; 11 family contacts; 17 hospital or laboratory personnel exposures to meningococcic infection; 48 cases of meningitis caused by organisms other than the meningococcus; 102 cases of febrile disease other than meningitis; 46 cases of gonococcic infection;* and 165 unselected serums sent to the laboratory for the Wassermann test.†

Method. The cultures used to prepare the test antigens were standard stock culture strains. These included Group I strains 331 and 1027,‡ Group II strain 36,§ and Group II-alpha strains 1166‡ and 3855.§ Antigens for each of these strains were prepared by washing the 5- to 6-hour growth of well encapsulated meningococci from Miller's medium[2] pint blake bottle slants, heating immediately in a water bath at 65°C for one hour, and diluting to a density of 2×10^{-9} cocci per ml in 0.5% phenolized, buffered saline solution.[3] The diluted antigens were stored

[1] Lingelsheim, W. von, *Klin. Jahnb. Jena*, 1906, **15**, 373; *Z. f. Hyg. u. Infectionskrank.*, 1908, **59**, 457; Elser, W. J., and Huntoon, F. M., *J. Med. Res.*, 1909, **20**, 371; Forster, M., and Gaskell, J. F., *Cerebro-Spinal Fever*, Cambridge, 1916, p. 105; Worster-Drought, C., and Kennedy, A. M., *Cerebro-Spinal Fever*, Black, London, 1919; Lewis, Thomas, Smith, H., and Dingle, J. H., *J. Clin. Invest.*, 1943, **22**, 361.

* Obtained through the courtesy of Miss Agnes C. Hamann of the Diagnostic Laboratory, New York City Department of Health.

† Obtained through the courtesy of Miss Emilie E. Weisse of the Wassermann Laboratory, New York City Department of Health.

‡ Obtained from the National Institute of Health, Bethesda, Maryland, through the courtesy of Dr. Sara E. Branham.

§ Obtained from the Division of Laboratories and Research, New York State Department of Health, Albany, New York, through the courtesy of Miss Sophia M. Cohen.

[2] Miller, C. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 989.

[3] Simmons, J. S., and Gentzkow, C. J., *Laboratory Methods of the United States Army*, 1935, 4th Edition, p. 567.

at 5°C. The specificity and agglutinability of each lot of cells was checked by testing them with homologous and heterologous strain rabbit antiserums, and normal human serum. Only such cells were used which were agglutinated by homologous strain rabbit antiserum to titer, and which did not show spontaneous agglutination in 0.85% buffered saline solution, or agglutination in normal human serums in titers exceeding 1-32. Stored cells were tested at bi-weekly intervals to check possible deterioration. Sufficient antigen to last approximately 2 months was prepared in each batch.

Five-tenths ml of serum dilution was placed in 12 x 75 mm test tubes, to which 0.5 ml of antigen was added. Serum dilutions were made in buffered saline solution at pH 7.2. Each serum dilution was increased twofold. The range of final dilutions of 1:8 to 1:512 was modified as the study progressed to 1:50 to 1:1600, so that normal agglutinins would be excluded, and a definite endpoint of the test serum would be obtained.

Methods of Incubation. The standard method of incubation at 37°C for 2 hours, followed by overnight storage at 5°C was the final determination of the serum titer and was used on every serum tested. In addition to this, 2 other methods of incubation were investigated. Six hundred and twenty-eight serums, representative of all the types studied. were centrifuged at high speed for 15 min. immediately after the antigen was added to the serum dilutions.[4] Four hundred and thirty-two (75%) of the tests treated in this way gave identical readings after immediate centrifugation as these same tests did following subsequent incubation at 37°C for 2 hours followed by storage overnight at 5°C. Ninety-one of the remaining 196 (25%) showed slightly higher readings after immediate centrifugation and 105 showed lower readings after immediate centrifugation than they did after incubation and storage in the standard manner. The third method was to delay centrifugation until after the tests had been incubated for 2 hours at 37°C. A group of 414 serums representing all types were tested in this manner. Only 12 (3%) of these showed variation in the readings made after centrifugation and the final overnight reading. The choice of method used for the incubation of the agglutination test was therefore one of expediency.

Reading. Large or medium clumps with a clear supernatant were considered 4+; large or medium clumps in a slightly cloudy fluid 3+; definite clumps in a cloudy fluid 2+; and a few fine clumps in a cloudy fluid 1+. The titer of the test serum was considered as the last tube showing definite clumping (2+). Care had to be taken when the observations were made on the centrifuged tests, since the packing of the cells might appear like clumping when they were first shaken. This was avoided by making a careful comparison with the control suspensions of unsensitized cells. After vigorous shaking, the clumps in the control and negative tubes disappeared, while the clumps in the tubes containing agglutinins persisted, although they became smaller.

Results. The range of normal agglutinins and the specificity of the tests were determined from the results of tests carried out on the first 50 serums of each of (a) the control group of unselected serums sent to the laboratory for the Wassermann test; (b) the group of serums from patients suffering from febrile diseases other than meningococcic infection; and (c) the group of serums from cases of bacteriologically proved meningococcic infection. These results are summarized in Table I. Since none of the serums in the control groups showed the presence of agglutinins in titers above 1-64, agglutinin titers of 1-128 or over were considered significant in indicating the presence of specific antibody, while those up to and including 1-64 were considered within the normal range.

It has been possible to demonstrate agglutinins for one or more of the types of meningococci used in this test in 195 of 276 patients suffering from clinically typical meningococcic infection. In 48 instances it was necessary to test 2 or more specimens of blood at intervals of from 3 to 7 days to obtain agglutinins in significant titers. In fact, failure to demonstrate agglutinins in 72 of the remaining 81 cases may be attributed to the fact that only one serum was tested from 0 to 8 days after

[4] Gates, F. L., *J. Exp. Med.*, 1921, **35**, 63.

TABLE I.

Comparison of Agglutinin Titers in a Control Group of Unselected Serums Sent for the Wassermann Test, a Control Group of Serums Obtained from Patients Suffering Febrile Diseases Other Than Meningococcic Infection, and a Group of Bacteriologically Proved Cases of Meningococcic Infection.

Serum titer	Serum sent for Wassermann test		Serums from cases of febrile disease other than meningococcic infection		Bacteriologically proved cases of meningococcic infection	
	No.	%	No.	%	No.	%
1-512	0	0	0	0	17	34
1-256	0	0	0	0	12	24
1-128	0	0	0	0	6	12
1-64	0	0	6	12	8*	16
1-32	4	8	15	30	4†	8
1-16	17	34	2	4	0	0
No agglutination	29	58	27	54	3†	6
Total	50	100	50	100	50	100

* Six instances only one test serum available.
† Only one test serum available.

onset. No agglutinins were found in the serums of cases of meningitis caused by organisms other than the meningococcus, in the serums of the group of 11 family contacts, or in the serums of 100 of 102 cases of other febrile diseases. It should be noted, however, that agglutinins in significant titers were demonstrated in 6 of 17 healthy hospital or laboratory personnel presumably exposed to meningococcic infection; in 11 of 46 instances of gonococcic infection; in 1 of 2 cases of Rocky Mountain spotted fever; and in 1 of 2 cases of staphylococcic septicemia. The significance of positive findings in cases not due to meningococcic infection merits further investigation.

It is of interest to note that Mayer and Dowling obtained[5] essentially the same results using freshly isolated strains and the centrifuge-agglutination method without further incubation at 37°C.

Summary and Conclusions. The agglutination test, using standardized, type specific, heated and phenolized antigens, prepared from stock culture strains, representative of the prevalent types of meningococci, offers a simple method for the demonstration of agglutinins in the serums of individuals suffering from meningococcic infection. The test may be useful as an additional diagnostic procedure in cases where other laboratory confirmation is lacking.

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5 Mayer, R. L., and Dowling, H. F., 1944, personal communication.