In view of the fact that one could be tempted to employ the neutralization test to demonstrate the existence of a past infection, it was of interest to compare the 50% endpoint neutralizing antibody titers against epidemic typhus obtained in late convalescent specimens from proven cases of epidemic typhus fever with those found in specimens from cases of Rocky Mountain spotted fever. The results are given in Tables II and III.

If we accept the figure 40 as representing the lowest 50% endpoint neutralizing titer (case 6243—strain isolated) which occurred in late convalescence in our series of epidemic typhus fever cases, it is observed that 9 of the 16 specimens studied from cases of Rocky Mountain spotted fever gave higher titers. As already pointed out three of the Rocky Mountain spotted fever specimens gave titers of epidemic neutralizing antibody as high as or higher than were found in late convalescent specimens from cases of epidemic typhus fever. These results must be taken into consideration if the neutralization test is used for survey purposes.

*Conclusion.* Serological studies on convalescent specimens from cases of Rocky Mountain spotted fever show the presence of epidemic and murine agglutinins and epidemic neutralizing antibodies although there is no evidence of cross complement fixation. This evidence fits in with the observations of Castaneda and Silva and those of Parker, and indicates that there is a serologic relationship between epidemic typhus fever and Rocky Mountain spotted fever. The data presented in this paper must be considered in evaluating the results obtained with rickettsial agglutination or the neutralizing antibody when these tests are used as diagnostic procedures.

## 14804

## pH of Nasal Mucosa of Some Laboratory Animals.

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The susceptibility of individuals to infections of the respiratory tract shows seasonal variations. This also appears to be true of some of the laboratory animals. A number of physiological factors are involved in the defense mechanism against bacterial and viral invasions, and in determining the severity of the infection. Of these factors, the pH of the nasal mucosa may play a role. There is disagreement as to what constitutes the normal pH of nasal mucosa. Using a glass electrode *in situ,* Fabricant[1] reported a surprisingly low average value of 6.2 for man with large fluctuations. Nungester and Atkinson[2] pointed out several errors in the method used by Fabricant. Their determinations yielded a value of pH = 7.0. Neither investigator recorded the temperature at which the readings were made. They also introduced a saline bridge without testing the effect of the additional boundary potentials. The effect of these boundary potentials has, however, been shown to be small.[3]

Hamsters were selected for the study of pH of nasal mucosa because the animals are susceptible to some viral diseases. Several readings were also made using rats and ferrets. This article deals with normal values. Special glass electrodes were constructed so that the entire glass membrane would be in contact with the nasal mucosa. The saturated calomel cell made direct contact with the animal through an asbestos-fiber wick.

*Methods.* The glass electrodes were made

[1] Fabricant, N. D., *Arch. Otolaryng.,* 1941, **34**, 150.

[2] Nungester, W. J., and Atkinson, A. K., *Arch. Otolaryng.,* 1944, **39**, 342.

[3] Voegtlin, C., Kahler, H., and Fitch, R. H., *Nat. Inst. Health Bull.,* 1935, **164**, 15.

by sealing a membrane of Corning 015 glass to a glass of high electrical resistance, Corning 881, as described by Kahler and Robertson.[4] For use in hamsters and rats, the membrane was 6-8 mm in length and 1 mm in diameter. The internal electrode consisted of a platinum electrode in a saturated solution of quinhydrone in 0.1 *N* hydrochloric acid, which was renewed daily. The electrode was connected to a Beckman pH meter with a shielded cable. The saturated calomel electrode was the asbestos wick type, and made direct contact with the animal so that no additional junction potentials were involved. The whole assembly was kept in a grounded metal box. Measurements were made in an air-conditioned room.

The hamsters were anesthetized intraperitoneally with 8.3 mg of sodium pentobarbital for each 100 g of animal weight. The hamster was laid on its back, and the glass electrode inserted to the full length of the membrane. In the first series of experiments the contact of the reference electrode was just above the teeth on the outside of the mouth. It was, however, found that more uniform readings resulted when the calomel electrode made contact in the roof of the mouth at the base of the teeth. The difference was less than 0.1 pH unit. All readings were made with the temperature compensator set at 35°C. The electrodes were standardized and frequently checked at room temperature with buffers of pH 4.00 and 7.00. The initial pH reading was usually high. It reached an equilibrium value in 5 to 10 minutes, and usually remained constant for about 5 minutes. Prolonged contact gave further changes. These cannot be considered significant as a film was deposited on the electrode from the drying of nasal secretions. This interfered with the uniform wetting of the electrode membrane. After removing the electrode from the nostrils it was washed and checked with the buffers.

To determine the nasal temperature a galvanometer scale was calibrated[5] using a copper-constantan thermocouple in a glass tube similar to those used in the construction of the glass electrode. A period of 5-10 minutes was required to reach an equilibrium value, which corresponded to the time required for the pH measurement to become constant. The hamsters had a nasal temperature of 32-33°C if measured with the thermocouple in the glass tube. Inserting the bare thermocouple directly into the naris gave a value about one degree higher. These values cannot be considered as absolute as the thermocouple cannot be used under conditions identical to those under which it was calibrated.[5] In measuring the pH no difference was observed whether the temperature compensator of the meter was set at 33 or 35°C. The latter setting was used in all determinations.

*Results.* In the first series 26 pH measurements were made on 10 hamsters. The reference electrode was at the base of the upper teeth on the outside of the mouth. The mean and mean deviation for the pH at 35°C for this series was 7.10 ± 0.129 with 65% of the values falling within these limits. Another series of 56 determinations in 12 different hamsters (calomel electrode at the base of the teeth in the roof of the mouth) gave a value of pH 7.19 ± 0.100, with 61% of the readings within these limits. The lower and upper values in this series were 6.93 and 7.42 respectively, with only one reading falling below 7.00. The pH values at 35°C for 3 rats were found to be 7.25, 7.28, and 7.30, and for 4 ferrets, 6.43, 6.75, 6.85, and 7.01. The ferrets were difficult to work with as their movements caused fluctuations in the readings.

The values obtained for hamsters agree well with those of Nungester and Atkinson[2] for the normal pH of the nasal mucosa of man. It is recognized that the readings obtained are not absolute values. Unknown boundary and skin potentials, inability of keeping the whole electrode system at constant temperature, and other errors of the electrode system make it impossible to obtain absolute values at the present time.

In addition to errors involved in making measurements, the Sörensen pH scale cannot at present be precisely defined for solutions other than pure water.[6] Many authors assume

[4] Kahler, H., and Robertson, W. v. B., *J. Nat. Cancer Inst.*, 1943, **3**, 495.

[5] Sheard, C., *Am. J. Clin. Path.*, 1931, **1**, 209.

[6] Nutting, P. G., *J. Franklin Inst.*, 1943, **236**, 573.

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.

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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 $2 \times 10^{-9}$ 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.