M. Feinberg, in charge of the Allergy Clinic, and the control sera were obtained through the courtesy of Dr. O. E. Hepler, of the Clinical Pathology Laboratory, who also supplied the sheep cells and amboceptor used in the titrations. The complement titrations were carried out on the same day as the routine Wassermann tests, and the cells and amboceptor used had been titrated with guinea pig complement. The control series comprised a large number of sera (more than a hundred), which were being used for other experiments.

Veil and Buchholz[6] found the complement titer of normal blood to range between 0.02 cc. and 0.06 cc., with an average value of 0.05 cc. A similar titer was found for the control sera of the present series (0.02 cc. to 0.07 cc., with an average of 0.05 cc.), and the allergic sera showed a similar range and similar average value (Table I).

Conclusion. The amount of complement present in the blood of allergic patients of this series (18 cases) was similar to that in the control sera from persons coming to the general medical clinic.

8003 P

A Study of Experimental Meningococcal Infection. I. Method.

C. PHILLIP MILLER. (With the technical assistance of Ruth Castles.)

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It was reported[1] that mice could be infected by intraperitoneal inoculation with small numbers of virulent meningococci suspended in a solution of mucin. The mucin used in those original experiments was a commercial product which is prepared from hog's stomach and marketed for the treatment of gastric ulcer. The strains of meningococci employed had been freshly isolated from the spinal fluids of patients suffering from epidemic meningitis, and of these strains the 2 which proved lethal in highest dilution were those which had been obtained under conditions that permitted initiation of the experimental infection in the mouse most quickly after aspiration of the spinal fluid.

When work on this problem was resumed after several months,

[6] Veil, W. H., and Buchholz, B., *Klin. Woch.*, 1932, **11**, 2019.

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

the minimal infecting inocula proved to be much larger than they had been in the preceding spring and summer. To explain this variation, attention was directed to the meningococci, the mice, and the mucin. In the case of each lay a possible source of our difficulty because the following changes had occurred:

1. Our cultures had been carried for a considerable length of time on artificial media without transfer to mice. Efforts to enhance the virulence by mouse passage were unavailing, and fresh strains of meningococci could not be secured because of the low incidence of epidemic meningitis in Chicago at the time.

2. Mice were difficult to obtain and came mostly from dealers who had not previously supplied us; presumably, therefore, from different stocks.

3. A new lot of mucin was being used as our initial supply had been exhausted. Several lots obtained from the laboratory which had furnished it and samples from the 2 other manufacturers who prepare mucin commercially were equally ineffective in facilitating experimental infection with small numbers of organisms. At length, on the assumption that the process of purification might "denature" the mucin or destroy some unrecognized ingredient essential to our purposes, the laboratory which had originally supplied us was requested to make up a special lot of mucin.* This proved to be wholly satisfactory and was used in the experiments described below.

Preparation of the mucin suspension. A 5% suspension is made up as follows: Onto the weighed quantity of mucin is poured enough distilled water to moisten it thoroughly. It is allowed to stand half an hour. Then the sticky mass is stirred and rubbed free of solid lumps. Stirring continues as distilled water is slowly added until the final concentration of 5% is reached. The reaction of suspension should be about pH = 5. It is placed in suitable containers and autoclaved at 15 lb. pressure for 15 minutes. After cooling, the reaction is adjusted with normal sodium hydroxide to pH = 7.3 and glucose is added to a concentration of 1%. After incubating one day to insure sterility the suspension is ready for use. As the product now being used contains particles of sufficient size to occlude fine hypodermic needles, it has been found convenient to withdraw the supernatant from this coarse sediment which rapidly settles to the bottom of the container and is easily visible to the naked eye. The supernatant, hereinafter designated the "mucin

*For this material the author is indebted to Dr. David Klein of the Wilson Laboratories, Chicago. It may be obtained under the label "Granular Mucin."

suspension" tends itself to settle on standing, particularly in the cold, and should be thoroughly mixed by gentle agitation before using, in order to insure its uniform composition in all experiments.

Stock cultures of meningococci are maintained on solid media consisting of equal parts of meat infusion broth and buffer solution (containing 6.6 gm. of $Na_2HPO_4.12H_2O$ and 0.83 gm. of $NaH_2PO_4.H_2O$ and 8.8 gm. NaCl per liter), 1% dextrose, 1% peptone, 1% corn starch and 2% agar. Its reaction is pH = 7.3 to 7.4. Inoculated agar slants are kept stoppered with corks. Although stock cultures are transplanted only twice a week, a strain which is to be used for mouse inoculation is subcultured at least twice at daily intervals before the final culture is inoculated. The organisms to be used are grown on the same medium for only 5 hours. They are then washed off with a few cubic centimeters of saline, the suspension thoroughly mixed and diluted with saline to a density which experience has shown to contain approximately $5\text{-}10 \times 10^9$ organisms per cc. Their numbers are determined by the Wright method, the smears being made at once, but the actual counting postponed until the completion of the animal inoculations.

The bacterial suspension is then titrated by progressive ten-fold dilution in the suspension of mucin. Mice are injected intraperitoneally with 1.0 cc. amounts as soon as possible after the dilutions have been prepared in order that their estimated bacterial content shall be altered as little as possible either by multiplication or death.

By this method a lethal infection can be initiated in the mouse by the intraperitoneal inoculation of a very few organisms, with our most virulent strains, approximately 10.

8004 P

A Study of Experimental Meningococcal Infection. II. Course of Infection.

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Mice infected intraperitoneally by the method described in the preceding communication[1] rapidly sicken and die within a period of 12 to 48 hours, depending on the number of meningococci injected.

[1] Miller, C. Phillip, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1136.