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.

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

From the Department of Medicine, University of Chicago, and the A. B. Kuppenheimer Research Foundation.

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.

The cellular and microbic contents of the peritoneal cavities of the mice in several series were studied at intervals of about 2 hours. A drop of exudate was aspirated by means of a fine hypodermic needle inserted into the peritoneal cavity and examined microscopically in stained film preparation. As a check on this method the entire peritoneal exudate was withdrawn from mice sacrificed at corresponding times during the course of their infection and mixed and examined by means of cultures and smears. The results of these observations may be summarized as follows: Cells did not appear in the peritoneal exudate in any considerable number until about 4 hours after inoculation, even when very heavy suspensions of organisms had been injected. From that time on the number of cells increased rapidly. Approximately two-thirds of them were polymorphonuclear leucocytes and one-third large and small cells with a single nucleus and various amounts of cytoplasm. No special methods were employed to differentiate these cells. As the infection progressed an increasing fraction of the cells showed evidences of degeneration.

Following inoculation with small or moderately large numbers of meningococci evidence of multiplication was seldom obtained for several hours. After 4 to 6 hours, however, the number of organisms increased rapidly and apparently steadily until death occurred. When the infection was initiated by injection of relatively massive inocula the multiplication seemed to begin earlier. Irrespective of the number of organisms inoculated very few were seen within leucocytes at any stage of the infection when the strain employed was a highly virulent one. The exudates from mice infected with less virulent strains, however, contained an appreciably larger proportion of polymorphonuclears in which diplococci were visible. In other words, the extent to which phagocytosis occurred was apparently related to the virulence of the infecting organism rather than to the numbers of individuals introduced into the peritoneal cavity.

Invasion of the blood stream was found to occur very shortly after intraperitoneal inoculation with large numbers of organisms. This point was investigated by culturing on the surface of freshly poured blood-agar plates a drop of blood drawn into a fine capillary pipette from the freshly cut end of the tail. The method was checked by culturing the heart's blood of mice sacrificed from time to time during the course of their infection. When the intraperitoneal inocula exceeded a million organisms, cultures of blood drawn within 15 minutes were positive, and the bacteremia persisted until death. Inoculations of a few hundred thousand organisms were

followed by cultures which did not become regularly positive for one or 2 hours. Still smaller inocula failed to produce a sustained bacteremia for 4-12 hours, though occasionally a "weakly positive" culture (*i. e.,* one or 2 colonies from a drop of blood) was followed by several negative ones. In the case of mice infected with very few organisms occasional cultures were "weakly positive" early in the course of the infection. All cultures of the blood of moribund mice were "strongly positive", *i. e.,* showed many colonies or a confluent growth on the plates. In summary, therefore, these observations indicate that transitory invasion of the blood stream may occur at any time during the course of the infection, but that the time at which persistent bacteremia begins is related roughly to the number of organisms with which the infection is initiated.

8005 P

A Study of Experimental Meningococcal Infection. III. Effect of Anti-bacterial Immune Serum.

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

From the Department of Medicine, University of Chicago, and the A. B. Kuppenheimer Research Foundation.

The effect of immune sera on experimental meningococcal infection in the mouse was studied to determine: (a) its protective action, when administered before inoculation, and (b) its ability to alter the expected outcome of an infection when administered during its course.

Sera were obtained from rabbits immunized by intravenous injections of living meningococci grown on solid media. Therapeutic sera (most of them "concentrated") prepared by a number of different commercial firms were purchased in the open market.*

Preliminary experiments showed that serum administered subcutaneously afforded less protection than corresponding doses given intravenously or intraperitoneally, these 2 being equally effective. As the intraperitoneal route was much less time-consuming it was used in all experiments herein reported. It was thought for a time that the preservatives added to commercial therapeutic sera might

* In addition 2 lots of concentrated sera, one prepared with and one without preservative (Merthiolate), were very kindly supplied by Mr. W. A. Jamieson, Director, Biological Division of the Lilly Research Laboratories, Indianapolis.