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.

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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.

exert a bacteriostatic action on the organisms injected into the peritoneal cavity 30 minutes later, but this supposition was proved to be unwarranted by the observation that the injection of such preservatives in concentrations considerably higher than those customarily employed failed to afford any detectable protection.

Dilutions of the sera were made with normal saline and were injected intraperitoneally in 0.5 cc. amounts approximately one-half hour before inoculation. Control mice received 0.5 cc. of saline at the same time. The inoculations were made by intraperitoneal injection of 1 cc. amounts of the suspensions of meningococci in mucin, titrated by ten-fold dilution, according to the method described in the first paper.[1] Although the weights of mice used varied from 18 to 24 gm., all of the animals in any given experiment weighed within 1 gm. of each other, a condition which placed a limitation on the size of the experiments.

Serum diluted 1:16 or less protected mice against inocula 100,000 to 10 million times the M.L.D. Serum diluted 1:2,000 protected against 100 to 10,000 M.L.D. Within this range protection was roughly proportional to the dilution of serum. Dilutions higher than 1:2,000 afforded inconstant protection against even small inocula. Among the few strains thus far studied, no evidence of strain specificity, as regards protection, has been encountered.

The results of protection experiments with commercial "concentrated" therapeutic serum indicate a degree of protection approaching, but not quite as high, as that effected by our rabbit serum.

To determine the duration of passive protection mice were injected with 0.5 cc. of quite a low (1:4) dilution of immune serum and tested, half of them 2 days, the rest 8 days later. They survived infection with an inoculum 100,000 times the lethal one.

The effect of immune serum on the experimental infection after its inception was studied by injecting the serum intraperitoneally into mice at different times after they had been inoculated with numbers of organisms which the controls showed to be varying multiples of a lethal inoculum. The results show that even relatively late in the course of the infection mice can be spared a fatal outcome by the administration of sizeable doses of serum.

Summary. Rabbit antimeningococcus immune sera as well as commercial therapeutic sera in high dilutions protect mice against infection with virulent meningococci. In comparatively low dilution they exert a favorable action on the course of the experimental in-

[1] Miller, C. Phillip. In press.

fection even when administered relatively late in its course. The mouse protection test is regarded as a more reasonable method than those at present employed for the standardization of therapeutic antimeningococcus sera.

8006 P

Experimental Freezing: Bleeding Volume, General and Local Temperature Changes.

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The bleeding volume in control dogs was found to be 58.6% of the calculated blood volume (one-thirteenth of the body weight) by Roome, Keith, and Phemister[1] and 53.4% by Harkins.[2] On the other hand, the former authors found that in shock due to trauma to an extremity, hemorrhage, plasmapheresis, and intestinal manipulation, the bleeding volume was greatly reduced, averaging 21.8% and in burns the latter author found it to average 20.3%. In another series of experimental burns it was found that in 6 burned animals in which the blood pressure was allowed to fall near a so-called shock level (50 to 82 mm. of Hg.) before the bleeding volume was determined, the bleeding volume averaged 26.3%. If, however, the bleeding was done before the blood pressure had fallen markedly (102 to 130 mm. of Hg.), the bleeding volume was already markedly reduced, averaging 31.4%.

The present work was undertaken to determine the bleeding volume in experimental freezing. Portions of the bodies of dogs were frozen by solid carbon dioxide under complete anesthesia (maintained till end of experiment) as described in previous papers[3] and the results shown in Table I. It is seen that in all instances the bleeding volume was below the normal values but the decrease was not quite as marked as in burned animals. In the first 3 experiments where the bleeding volume was only slightly reduced, the

[1] Roome, N. W., Keith, W. S., and Phemister, D. B., *Surg. Gynec. and Obstet.*, 1933, **56**, 161.

[2] Harkins, H. N., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 3.

[3] Harkins, H. N., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 432.