obtained by a previous investigator.[1,2] 3. The immunity produced by this method is effective against the lung and intestinal phases, and possibly the skin phase, of the parasite, in the same manner as those reported by previous investigators after active (by prior infection) and passive immunization against this parasite.[5,6,7]

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Use of Chicken Serum in the Species and Type Identification of Neisseria.*

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The Commission on Meningococcal Meningitis, of the Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, has been concerned with the problem of identifying and subclassifying meningococci in connection with the occurrence of meningococcic meningitis in the Army. Certain difficulties were encountered in the preparation and use of monovalent sera. Rabbits are extremely susceptible to the toxic effects of the meningococcus and small, carefully graduated doses must be employed. A long period of time is usually required and animals frequently react poorly. The sera obtained are not sharply specific and will commonly show group agglutinins with the other types. Furthermore, with the technics usually recommended[1] the agglutination reactions are difficult to interpret because of low titer differences and fine clumping which must be read with a magnifying glass.

It was decided to investigate the agglutinin response of other animals and of fowl. Five young dogs were given intravenous injections of large numbers of living meningococci of the various types once a week for a period of 6 weeks. Although they tolerated the toxic effect, sera taken during and after this period contained no agglutinins. On the other hand, 5 chickens treated in a like manner not only exhibited similar tolerance but furnished sera with highly specific agglutinins. This lead was followed with gratifying results, and a preliminary report of the procedure is herewith presented.

Ten chickens of the Rhode Island Red variety were injected with *N. intracellularis* (Groups I, II, Types II alpha and IV), and with *N. gonorrhoeae* at weekly intervals, using the wing vein. The schedule was so arranged that the chickens received at the beginning of the first and second weeks 2 billion cocci, the third and fourth 4 billion, and the fifth and sixth 6 billion. They were bled from the heart at the beginning of the fourth and seventh weeks, and the titer of the agglutinins in each serum was ascertained for each of the strains employed.

The agglutination technic adopted was a modification of that suggested by Noble.[2] The antigens were prepared by suspending the organisms from a 5- to 24-hr culture in a physiological saline solution containing 0.05% potassium cyanide and standardizing to match the No. 8 tube of the McFarland nephelometer. The potassium cyanide was added because it was thought that the resulting suspensions were more homogeneous and did not settle so rapidly. The chicken

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1 Branham, Sara E., *The Meningococcus. Diagnostic Procedures and Reagents*, 1st ed., Am. Pub. Health Assn., 1941, 60-84.

2 Noble, A., *J. Bact.*, 1927, **14**, 287.

TABLE I.
Rapid Agglutination Titers of Chicken Sera.*

Chicken No.	Strains employed in immunization	Strain No.	Fourth week Agglutination titers					Seventh week Agglutination titers				
			MC17	MC382	MC19	MC21	GC44	MC17	MC382	MC19	MC21	GC44
	Group I											
26	Meningococci	MC17	1/32	0	0	0	0	1/16	0	0	0	0
27	" "	MC17	1/32	0	0	0	0	1/32	0	0	0	0
	Type II alpha											
42	Meningococci	MC382	0	1/32	0	0	0	0	1/32	0	0	0
43	" "	MC382	0	1/32	0	0	0	0	1/32	0	0	0
	Group II											
30	Meningococci	MC19	0	0	1/64	0	1/8	0	0	1/64	0	1/16
31	" "	MC19	0	0	1/64	0	1/8	0	0	1/64	0	1/16
	Type IV											
32	Meningococci	MC21	0	0	0	1/128	0	0	0	0	1/32	0
33	" "	MC21	0	0	0	1/128	0	0	0	0	1/32	0
24	Gonococci	GC44	0	0	0	0	1/32	0	0	0	0	1/32
25	"	GC44	0	0	0	0	1/32	0	0	0	0	1/32

*Serum titer is that at which 3+ or 4+ agglutination occurred after 3 minutes agitation and represents the degree of dilution after the addition of the antigen but before the final addition of saline.

sera were diluted with physiological saline 1:1, 1:2, 1:4, 1:8, 1:16 and 1:32, and 0.1 ml of each dilution was placed in the bottom of the small tube. To this was added 0.1 ml of the antigen, doubling the initial serum dilution. The mixture was gently rocked for 3 min and then, to facilitate reading, was further diluted by adding 0.8 ml of saline. The reactions were classified immediately as complete, almost complete, partial, slight, or no agglutination and recorded as 4+, 3+, 2+, 1+ or 0, respectively.

The agglutination titers of the various chicken sera are presented in Table I. The titer given is the highest dilution in which agglutination was almost or quite complete and is recorded in terms of the dilution of the serum after the addition of the antigen and before the final dilution with saline.

The sera prepared by injecting chickens with the various meningococcal and the gonococcal strains agglutinated only their homologous suspensions with one exception. That obtained after injections of Group II meningococci flocculated the gonococcal suspension, although not in as high titer as the homologous strain suspension. The reactions were sharp, with no other cross-agglutinations, even in the low dilutions. The organisms formed firm flocs which could be seen without magnification.

Discussion and Summary. Highly specific and potent agglutinating sera for the various types of *N. intracellularis* and for *N. gonorrhoeae* were quickly and readily obtained from chickens following relatively large intravenous injections of living organisms. Their employment in a rapid agglutination technic affords a dependable means of identification of the various Neisseria. This method does not require incubation at 37°C and storage in the icebox overnight. The reaction occurs at room temperature, is read immediately, and the agglutinated organisms form clumps which can be seen easily. It is possible that the employment of chicken sera in the species and type identification of Neisseria will provide a better means of exploring the antigenic and epidemiologic relationships of this group of microörganisms.

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