

difference in the degree of reduction of the sheep hemagglutinin titer of sera from 10 patients with infectious mononucleosis by this anti-heterophile antibody rabbit serum. Although no antigenic heterogeneity among heterophile antibodies in sera obtained from different patients was demonstrable, the results indicate that heterophile antibodies are specific macroglobulins which differ from the other macroglobulins found in sera of patients with infectious mononucleosis.

We are grateful to Drs. J. Rigsby and J. Michaels for the patient sera.

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Received August 28, 1967. P.S.E.B.M., 1967, v126.

### Group-Specific Hemagglutination Test For *Neisseria meningitidis* Antibodies. (32593)

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The significance of human carriers of *Neisseria meningitidis* and meningococcal disease is obscured by the lack of an easily performed and specific serological test(1). None of the serological methods so far described for the serodiagnosis of meningococcal carrier status or disease has proven entirely satisfactory.

In the past several years, indirect or passive hemagglutination (PHA) has become one of the most widely used methods for measuring various antibodies. A number of techniques has been devised for the attachment of antigen to the erythrocyte (rbc) surface(2-9). The present report describes a modification of 1 of these(8) that resulted in an antigen from *N. meningitidis* which would adsorb to a sheep rbc. This rendered the sheep rbc agglutinable in the presence of antiserum homologous to the sensitizing antigen. Its use in the

PHA test for the measurement of *N. meningitidis* antibodies is also described.

**Methods. Antigen preparation.** Stock cultures of *N. meningitidis* groups A (Cl-4),\* B (16B6),\* C (PTS-5),\* Bo,† and 29E† were used to prepare antigens. One ml of an 8-hour Mueller-Hinton†(10) broth shake culture of each group was inoculated into 250 ml Mueller-Hinton broth and incubated for 18 hours at 37°C in a Psychrotherm Incubator shaker‡ (170-200 rotations/minute) with normal atmospheric air.

\* Obtained from Lt. N. Vedros MSC USN, NAMRU-1, Oakland, California.

† WRAIR strains provided by Lt. D. T. Kingsbury MSC USN, NAMRI, Bethesda, Maryland.

‡ Difco Laboratories, Detroit, Michigan.

§ New Brunswick Scientific Co., New Brunswick, New Jersey.

As a safety precaution, cultures were then inactivated with beta-propiolactone (1% final concentration) for 1 hour at room temperature and overnight at 6°C. The sediment was collected by centrifugation ( $600 \times g$ ), washed 3 times with 0.15 M NaCl (saline) and resuspended in 10 ml of saline. The suspension was adjusted to pH 11.0 with 1 N NaOH, incubated for 1 hour at room temperature and then readjusted to pH 6.5 with 1 N HCl. The suspension was precipitated with 5 volumes of absolute ethanol. The precipitate was collected by centrifugation ( $3000 \times g$ ) and resuspended in 20 ml saline. The insoluble residue was removed by centrifugation and discarded. The clear supernatant contained a soluble group-specific antigen which was used to sensitize sheep rbc.

**Sensitizing the erythrocyte.** Sheep rbc were collected in Alsever's solution and stored at 6°C for from 6 to 30 days. Equal volumes of a saline washed 2% rbc suspension were mixed with the antigen. The mixture was incubated in a 37°C water bath for 1 hour with occasional shaking. The rbc were then sedimented by centrifugation ( $200 \times g$ ), washed once with saline, and resuspended to an 0.5% suspension in 0.01 M phosphate buffered saline, pH 7.2, containing normal rabbit serum at a final concentration of 1:200 (NRPBS). The rabbit serum was absorbed with sheep rbc (0.1 ml packed washed sheep rbc/ml inactivated serum) to remove non-specific agglutinins.

**Determining sensitizing potency.** To determine the optimal sheep rbc sensitizing dilution of each antigen extract, varying concentrations of antigen ranging from undiluted to 1:800 were used for sensitization. These sensitized rbc were added to a homologous meningococcal grouping serum diluted from 1:2 to 1:512 (see PHA test procedure). The highest dilution of antigen causing agglutination of sheep rbc in the presence of 1 unit predetermined HA titer of homologous anti-*N. meningitidis* rabbit serum was considered to be 1 sensitizing unit. In the PHA test, 2 sensitizing units of antigen were used to insure adequate sensitization of the sheep rbc.

**The PHA test.** Increments of 2-fold dilutions of each inactivated (56°C for 30 min-

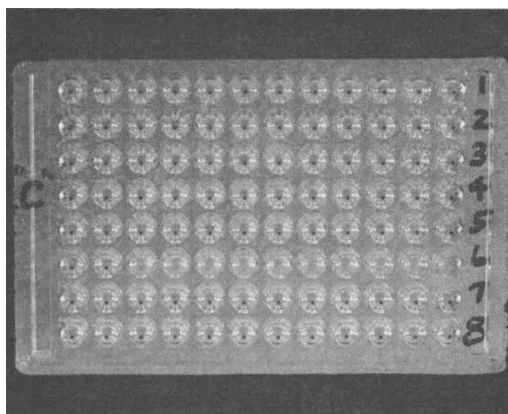


FIG. 1. A typical passive hemagglutination pattern using homologous and heterologous antisera. Sheep erythrocytes sensitized with *N. meningitidis* antigen group C. Two-fold dilutions of antisera from right to left of the microplate. No. 1 Anti-*N. perflava*; 2 Anti-*N. flavescens*; 3 Anti-*N. gonorrhoeae*; 4 Anti-*N. meningitidis* group Bo; 5 Anti-*N. meningitidis* group 29E; 6 Anti-*N. meningitidis* group C; 7 Anti-*N. meningitidis* group B; 8 Anti-*N. meningitidis* group A.

utes) test serum were made in 0.025 ml NRPBS diluent in Microtiter "V" plates.¶ One drop (0.025 ml) of the sensitized rbc was added to each well, carefully mixed, and incubated at 37°C. The tests were read when the cell control (containing 0.025 ml NRPBS and 0.025 ml test cells) formed a button (about 1 hour). The antibody titer was considered to be the reciprocal of the highest dilution of the serum that agglutinated 75% or more of the rbc. Serum controls using 0.5% suspension of non-sensitized sheep rbc and test sera were used to detect non-specific or Forssman antibody hemagglutination. Such sera were absorbed for 1 hour at room temperature with an equal volume of a 50% suspension of washed sheep rbc.

**Results.** Table I shows the high order of specificity obtained with various hyperimmune sera when sheep rbc were sensitized with homologous and heterologous meningococcal antigens. Prozones were never observed and cross-reactions were minimal (1:2 or less). A typical sensitizing potency determination is shown in Table II. Antigen extracts have ranged from 1:50 to 1:100 in sensitizing potency. This method of preparing an antigen and sensitizing rbc was re-

¶ Cooke Engineering Co., Alexandria, Va.

TABLE I. Specificity of the Passive Hemagglutination Antibody Test Using Various Antisera Against Homologous and Heterologous Sensitized Erythrocytes.

Antiserum	Erythrocytes sensitized with antigen from <i>N. meningitidis</i> group:				
	A	B	C	Bo	29E
<i>Anti-N. meningitidis</i>					
Group A	1:256	<1:2	<1:2	<1:2	<1:2
B	1:2	1:32	<1:2	<1:2	<1:2
C	<1:2	<1:2	1:256	<1:2	<1:2
Bo	<1:2	<1:2	<1:2	1:512	<1:2
F	<1:2	<1:2	<1:2	<1:2	1:512
<i>Anti-N. gonorrhoeae</i>	<1:2	<1:2	<1:2	<1:2	<1:2
<i>Anti-N. perflava</i>	<1:2	<1:2	<1:2	<1:2	<1:2
<i>Anti-N. flavescens</i>	<1:2	<1:2	<1:2	<1:2	<1:2

Antisera A, B and C lots Difco No. 499231, 496356 and 491435, respectively.

Antisera F, *N. perflava* and *N. flavescens* kindly supplied by Lt. N. Vedros MSC USN.

*Anti-N. gonorrhoeae* kindly supplied by Wm. Peacock, Jr., CDC, and Anti-Bo supplied by Lt. Cdr. L. F. Devine MSC USN, NAMRU-4.

TABLE II. Potency Determination of Homologous Meningococcal Antigens Used to Sensitize Sheep Erythrocytes.

Dilutions of homologous antigen used to sensitize sheep erythrocytes	Reciprocal of hemagglutination titer using <i>N. meningitidis</i> immune serum				
	Group A	Group B	Group C	Bo	29E
1:10	4096	128	1024	256	512
1:20	2048	128	1024	256	1024
1:30	2048	128	1024	256	512
1:40	2048	128	1024	256	512
1:50	2048	128	1024	256	256
1:100	1024	32	1024	64	128
1:200	32	2	512	8	32
1:400	<2	<2	16	<2	<2
1:800	<2	<2	<2	<2	<2

peated at least 6 times with 4 different cultures of each of the meningococcal groups A, B, C, Bo, and 29E without significant change of sensitization potency and without any change in specificity. The typical hemagglutination pattern shown in Fig. 1 illustrates that endpoints are easily recognized.

**Discussion.** This method of preparing a group-specific *N. meningitidis* sheep rbc sensitizing antigen has been shown to be easily reproduced with consistent specificity. The lipopolysaccharide of Gram-negative bacteria possesses erythrocyte sensitizing properties which, on mild alkaline hydrolysis, by heating, or a combination of the 2, enhances this property as much as 100- to 1000-fold(9). In our method, mild alkaline hydrolysis and alcohol precipitation produced *N. meningitidis* antigen extracts with sensitizing potency in dilutions ranging from 1:50 to 1:100 for one

sensitizing unit. Efforts to increase the agglutinative activity of the antigen by multiple sensitizations of the sheep rbc's proved no better than a single sensitization. These antigens have retained their sensitizing capacity and specificity, after storage at 6°C, for over 4 months. Sensitized rbc's have been shown to be usable for at least 1 week without loss of specificity or sensitivity. Further, it was found that sheep rbc's could be sensitized with at least 5 meningococcal antigens, either consecutively or simultaneously, and still retain their specificity and sensitivity(11).

In a large series of Naval recruits studied, where paired sera and bacterial samples were available, there was excellent agreement between the meningococcal PHA test and bacterial recovery(11).

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Received August 28, 1967. P.S.E.B.M., 1967, v126.

### Renal Lysozyme Levels in Mice Thymectomized at Birth.\* (32594)

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Neonatal thymectomy in mice induces a syndrome of wasting which closely resembles classical runt disease. The latter is a form of graft-versus-host (GVH) reaction produced by injecting neonates with adult lymphoid cells of a different histogenetic origin. The resemblance includes weight loss, lethargy, diarrhea, hyperplasia of the reticuloendothelial system with terminal lymphoid depletion, and death. Phagocytic activity as measured by elimination of carbon particles from the blood is increased in GVH disease(1) and in athymic runts(2). Furthermore, the immunologic competence both of athymic runts and of animals undergoing a GVH reaction is reduced (3). Recent work by Troup and Walford(4) indicated that a striking increase in renal lysozyme occurs in mice undergoing classical runt disease. In the present report we show that renal lysozyme is also elevated in thymectomized mice and that this elevation correlates with the runting phenomenon.

**Materials and methods.** Inbred C3H mice were employed. Each newborn litter was randomly divided into individuals to be either thymectomized or sham-operated. In addition, 2 litters were set aside as non-operated controls. Thymectomy was performed within 24 hours of birth by the method of Sjodin *et al*(5), with Fluothane anesthesia. Sham-operated mice were similarly treated except that the thymus was not actually removed.

All mice were weaned at 21 days of age, segregated according to sex into cages of from 1 to 4 mice, and maintained on standard mouse chow, supplemented for the first 10 days with wheat germ. Non-sterile water containing Tetracycline was given *ad lib*. A non-sterile, but clean environment was maintained.

The mice were weighed individually at weaning, at 6, 8, and 10 weeks of age, and at time of sacrifice. Thymectomized mice were sacrificed by cervical dislocation when characteristic symptoms of runting, including weight loss(6,7), developed. Diarrhea alone was not considered sufficient evidence of runting. Sham-operated and normal mice, and thymectomized mice without symptoms of wasting, were sacrificed along with the thymectomized wasted mice.

Immediately following sacrifice of a mouse both of its kidneys were removed, quick-frozen, and stored at  $-65^{\circ}\text{C}$ . The spleen was weighed and a gross autopsy examination was performed. Representative hematoxylin and eosin-stained sections of liver and spleen were prepared from individuals of various ages within experimental and control groups. Renal lysozyme activity was determined as reported previously(4).

For statistical analysis, only those mice exhibiting overt symptoms of wasting were considered runts. Animals thymectomized at birth that did not waste before sacrifice were considered thymectomized non-runts, and were treated as a separate group. The Fisher ratio

\* This work was supported by United States Public Health Service Research Grant No. HD-00534.