ministration may be related to this activity of serotonin.

1. Brodie, B. B., Pletscher, A., Shore, P. A., *Science*, 1955, v122, 968.
2. Perera, G. A., *J.A.M.A.*, 1955, v159, 439.

Received February 15, 1956. P.S.E.B.M., 1956, v91.

Separation of Antibodies in Syphilitic Rabbit Sera by Electrophoresis-Convection.* (22312)

WILTON E. VANNIER.† (Introduced by H. J. Magnuson.)
Venereal Disease Experimental Laboratory, U. S. Public Health Service, School of Public Health, University of N. Carolina, Chapel Hill.

The method of electrophoresis-convection has been used by Cann *et al.*(1-3) to obtain separations of a number of different types of antibodies. Their work suggested that it might be possible to separate the Wassermann reagin antibody from the antibody producing immobilization of spirochetes in the *Treponema pallidum* immobilization (TPI) test(4) and to relate these antibodies to electrophoretic serum components.

In this study a pool of syphilitic rabbit sera was fractionated by electrophoresis-convection and the resulting materials tested for the Wassermann reagin antibody by the VDRL flocculation test(5), tested for immobilizing antibodies in the TPI test and also tested for agglutinating activity(6). The fractions obtained were characterized by electrophoretic analysis.

Methods. The electrophoresis-convection apparatus used was that described by Raymond(7)‡. The operation of the apparatus was carried out in a coldroom at ca. 4°C. A 4 l bottle filled with buffer was placed in the buffer circulating system to increase the volume of circulating buffer to ca. 8 l. The increased buffer volume seemed desirable to minimize pH changes in the buffer during the course of the run. Protein concentrations were estimated by semimicro Kjeldahl nitrogen determinations and the factor 6.25 was used to convert nitrogen to protein. The relative composition of the serum fractions was determined by paper electrophoresis using the method developed by Grassmann and Hannig(8)§. The mobilities were determined by moving boundary electrophoresis using the Perkin-Elmer Model 38 Electrophoresis Apparatus. The determinations were carried out at a protein concentration of 0.3% in barbital buffer (pH 8.6, $\mu = 0.1$). The samples were simultaneously dialyzed for 48 to 72 hours against the same 2 l volume of barbital buffer. The specific conductance of the buffer after dialysis was used in the mobility calculations. The mobilities reported are the average of those calculated from photographs of the descending boundaries taken by the Longsworth scanning procedure, at 1, 1.5 and 2.0 hours. The VDRL slide flocculation test was used to measure the relative Wassermann reagin antibody content of the fractions. The serum fractions were not heat inactivated prior to testing. The TPI test with modifications(6) was carried out on serum fractions that had been diluted with normal rabbit serum (Pool I) to a final concentration of 2.0% of serum fraction protein and then heat inactivated at 56°C for 30 minutes. The samples tested were of the order of half serum

* The author wishes to thank Dr. Charlotte McLeod for performing the agglutination studies, Mr. George Cannefax for carrying out the TPI assays, Mrs. Barbara Stanley for nitrogen analyses and Mr. L. L. Roach for technical assistance.

† Present address: Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, Calif.

‡ Manufactured by E. C. Apparatus Co., New York City.

§ The paper electrophoresis apparatus and densitometer were obtained from E. Miltenberg Inc., New York City.

TABLE I. Serologic Analysis.

	TPI		Agglutination		VDRL			
	Protein conc., %	Titer*	Protein conc., %	Titer‡	Protein conc., %	Undiluted	1:2	1:4
Pool VI (syphilitic) undiluted	6.8	1: 940	6.77	1: 80	6.8	2+	1+	neg
Pool VI 2%	2.0	1: 340	2.0	1: 20	2.0	±	neg	"
pH 7.5 Top	2.0§	1: 820	2.0§	1: 80	5.9	4+	3+	"
7.0 "	2.0§	1: 1170	2.0§	1:160	2.8[5]	2+	neg	"
6.5 "	2.0§	1: 1300	2.0§	1: 80	3.4	±	"	"
6.0 "	2.0§	1:10000	2.0§	"	3.4	±	"	"
5.5 "	1.0§	1: 1920†	1.0§	" †	1.8[5]	neg	"	"
Pool I (normal) undiluted	6.2	neg‖	6.22	undil. 1+	6.2	"	"	"

* Dilution of serum protein to produce 50% immobilization of spirochetes.
† Titer on basis of a 2.0% solution.
‡ Dilution of serum protein to produce a 2-3 agglutination of spirochetes.
§ Serum fraction protein concentration.
‖ Negative.

fraction protein and half normal rabbit serum protein. All the TPI titers were obtained from a single assay of high sensitivity and are reported as the dilution of the sample necessary to produce 50% specific immobilization of the spirochetes. The same samples prepared for the TPI assay were tested for *T. pallidum* agglutination activity by the method of McLeod and Magnuson(6). The titers reported for comparison were obtained from a single test and are the greatest dilutions of the serum fractions that produced 2 to 3+ spirochete agglutination.

Experimental. The syphilitic serum used in this study was an aliquot of a serum pool from 105 rabbits that had had syphilis for 4½ to 7½ months. They had been inoculated intratesticularly with the Nichols pathogenic strain of *T. pallidum*. The pooled serum and the serum fractions were refrigerated at −10 to −20°C except when fractionated or tested. A total volume of 135 ml of the syphilitic rabbit serum was processed in three batches of 45 ml each. The serum was diluted with an equal volume of buffer which gave a final protein concentration of 3.4%. Fresh sodium phosphate buffer was used for each run. The sample was dialyzed against the buffer in which it was to be fractionated. During each fractionation a current of 0.1 ampere and 8 to 10 volts (field strength ca. 2 volts cm^{-1}) was passed through the system for 94 to 96 hours. Fractionations were carried out in sodium phosphate buffers of 0.1 ionic strength and at a series of pH's 7.5, 7.0, 6.5, 6.0, and 5.5. The first run was made at pH 7.5 and the "bottom" from this run was then fractionated at pH 7.0. The process was repeated through the 5 different buffers. The corresponding portions from the fractionation of each of the 45 ml aliquots were pooled before proceeding to the fractionation at the next pH. At the end of this procedure there were 5 "top" fractions and one "bottom" fraction.

Results. The "bottom," which consisted principally of albumin with a little globulin, gave negative TPI, agglutination and VDRL tests and was discarded. The relative com-

TABLE II. Electrophoretic Analysis.

	Relative composition, %*				Mobilities (cm^2 $volts^{-1}$ sec^{-1}) † × 10^5	
	Alb.‡	α-glob.	β-glob.	γ-glob.	β-glob.	γ-glob.
Pool VI	60 (67)	9 (11)	14 (10)	17 (12)		
pH 7.5 top	2	0	4	94		−1.2[6]
7.0 "	0	0	11	89		−1.4[2]
6.5 "	0	0	10	90	−3.5[2]	−1.6[3]
6.0 "	4	0	20	76	−3.6	−1.7[5]
5.5 "	8	6	30	56	−3.5[3]	−1.9

* Relative protein concentrations were estimated by paper electrophoresis except those values for Pool VI in parenthesis which were estimated by moving boundary electrophoresis (protein 1.7%, barbital buffer pH 8.6, $\mu = 0.1$).
† Mobilities were determined by moving boundary electrophoresis (protein 0.3%, barbital buffer pH 8.6, $\mu = 0.1$).
‡ Alb. = albumin; glob. = globulin.

position of the proteins in the 5 "top" fractions was determined by paper electrophoresis. It was found that they contained from 20 to 40% albumin. This indicated a poorer separation than had been previously reported for a similar electrophoresis-convection fractionation of rabbit serum(3). Serologic tests of the fractions showed little if any separation of antibody activities. Therefore each of the 5 "tops" was refractionated in the electrophoresis-convection apparatus using a buffer of the same composition as that in which it was originally separated. The 5 "bottom" fractions were discarded and the "top" fractions were concentrated by pervaporation, dialyzed against pH 7.0 phosphate buffer and the serologic tests carried out. In each of the tests a sample of the unfractionated serum, designated Pool VI, was run at the same protein concentration as the fractions being tested. The serologic results are shown in Table I. The relative composition of the serum fractions was determined by paper electrophoresis and the mobilities determined by moving boundary electrophoresis. The results of the electrophoretic analysis are presented in Table II.

Discussion. The electrophoretic results are in accord with those obtained previously by a similar fractionation of rabbit serum(3). There is a progressive increase in the mobility of the γ-globulin isolated at successively lower pH's and there is no detectable difference in the mobilities of the β-globulins obtained.

The VDRL test results indicate a relative concentration of the Wassermann reagin antibody in the fractions isolated at pH 7.5 and 7.0 and it is probable therefore that this antibody is associated with the slow γ-globulin. There is a relative concentration of the *T. pallidum* immobilizing activity in the fraction isolated at pH 6.0. The difference in titer between the pH 6.0 fraction and the fractions next higher in immobilizing activity corresponds to $2\frac{1}{2}$ to 3 tubes of 2-fold dilution and is believed to be significant. The most reasonable interpretation is to ascribe the immobilizing activity to components in the fast γ-globulin. The serum protein fractions were obtained by a method that produces separations on the basis of differences in electrophoretic mobility. The possibility exists that the differences in antibody activity measured were due to a fractionation of the α or the β globulin components. There was no evidence obtained for differences in mobilities among the α components or the β components of the various fractions. The more likely interpretation however is to associate the differences in antibody activities that have been demonstrated with the differences in mobilities of the γ-globulin fractions that have been measured.

There does not appear to be any significant concentration of agglutinating activity in any of the fractions. This may be a reflection of the importance of more than one antibody in the agglutination reaction(9).

Summary. An electrophoresis-convection fractionation of a pool of syphilitic rabbit serum has been carried out. Significant differences in the mobilities of the γ-globulin components of these fractions have been demonstrated. No such differences in the mobilities of the β-globulin components were found. TPI, VDRL and agglutination studies were carried out. A comparison of the serologic and electrophoretic data indicates that the Wassermann reagin antibody is concentrated in the slow γ-globulin component and the *T. pallidum* immobilizing antibody is concentrated in the fast γ-globulin component. There is no evidence for the concentration of *T. pallidum* agglutinating activity in any of the fractions.

1. Cann, J. R., Brown, R. A., Gajdusek, D. C., Kirkwood, J. G., and Sturgeon, P., *J. Immunol.*, 1951, v66, 137.

2. Cann, J. R., Brown, R. A., Kirkwood, J. G., and Hink, J. H., *J. Biol. Chem.*, 1950, v185, 663.

3. Cann, J. R., Campbell, D. H., Brown, R. A., and Kirkwood, J. G., *J. Am. Chem. Soc.*, 1951, v73, 4611.

4. Nelson, R. A., Jr., and Mayer, M. M., *J. Exp. Med.*, 1949, v89, 369.

5. Harris, A., Rosenberg, A. A., and Del Vecchio, E. R., *J. Ven. Dis. Inform.*, 1948, v29, 72.

6. McLeod, C. P., and Magnuson, H. J., *Pub. Health Rep.*, 1953, v68, 747.

7. Raymond, S., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 278.

8. Grassmann, W., and Hannig, K., *Hoppe-Seyler's Z. physiol. Chem.*, 1952, v290, 1.

9. McLeod, C. P., and Stokes, P. S., *Pub. Health Rep.*, 1955, v70, 379.

Received February 23, 1956. P.S.E.B.M., 1956, v91.

Serological Response to Japanese B Encephalitis Vaccine of Children and Horses Immune to St. Louis Virus.* (22313)

W. MCD. HAMMON, GLADYS E. SATHER, EDWIN H. LENNETTE, AND W. C. REEVES.

Department of Epidemiology and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pa., Viral and Rickettsial Disease Laboratory, State of California Department of Public Health, Berkeley, and the George Williams Hooper Foundation for Medical Research and School of Public Health, University of California, San Francisco and Berkeley.

In the spring of 1946 an opportunity was afforded one of us (W. McD. H.) to make serological tests on adult Japanese and American troops given a single injection (1 ml) of Japanese B encephalitis (JBE) mouse brain vaccine, then in current use in American troops. Japanese with elevated neutralizing antibody titers to JBE virus and without detectable complement fixing (C.F.) antibody prior to vaccination responded with elevated C.F. titers within 10 days after injection of a single dose of vaccine(1). This observation was subsequently confirmed by Sabin, Ginder, Matumoto and Schlesinger(2). On the other hand, American troops usually had neither neutralizing nor C.F. antibody responses after such an interval and only about 50% responded with significant neutralizing antibodies after the regular course of 3 injections; also, with rare exceptions, there was no C.F. antibody detectable at any time(1,3). This led us to wonder whether a rapid C.F. antibody response after an injection of JBE vaccine might be a specific means of determining whether an individual found to have JBE neutralizing antibody had been previously infected with JBE virus or whether such antibodies might be the result of an infection with St. Louis encephalitis (SLE), West Nile (WN) or another virus in this closely related group. In this connection, 2 of us (W. McD. H. and W. C. R.), while at the Hooper Foundation, had repeatedly observed in performing sero-diagnostic work for the arthropod-borne viral encephalitides that many humans and horses with SLE virus neutralizing and C.F. antibodies also had elevated serological titers to JBE virus. In some instances the titers to the latter agent were higher than those to SLE virus. Similar diagnostic rises occurred with both viruses. Obviously, if both viruses should be present in the same area this would pose a serious diagnostic problem. Also, as has already occurred in our experience and in that of others, antibodies for several of these viruses have been found in populations of other countries prior to isolation of any virus. Which virus gave rise to the antibodies has not always been obvious. The experiments reported in this paper were undertaken to see whether administration of JBE vaccine would permit of specific serologic differentiation for JBE virus infection.

Materials and methods. All sera after collection were frozen in rubber stoppered Pyrex glass tubes until tested. They were shipped with dry ice, then held at −25°C. All sera in a series from the same host and pertaining to one experiment were tested simultaneously. Neutralization tests were performed both by the intracerebral route (in 3 to 4-week-old mice) and by the intraperitoneal route (in

* These studies were conducted under sponsorship of Commission on Viral Infections, Armed Forces Epidemiological Board, and were supported in part by Office of Surgeon General, Department of Army, and by research grant C-481(C-7) from National Microbiological Institute of National Institutes of Health, Public Health Service.