gressed upon the 4-hour fasted body weight and the appropriate regression equations determined.

We are grateful to Mr. Raymond Neff for the computer analysis and to Miss Elizabeth Loutrell and Mr. James Richards for expert technical assistance. These studies were supported in part by a grant-in-aid from the National Institute of Arthritis and Metabolic Diseases (AM 02911), National Institutes of Health, US Public Health Service; and by the Fund for Research and Teaching, Department of Nutrition, Harvard School of Public Health.

1. Block, R. J., Bolling, D., The Amino Acid Composition of Proteins and Foods, 1951, C. C Thomas, Springfield, Ill.

2. Brody, Bioenergetics and Growth, 1945, Reinhold Corp., New York.

3. Brozek, J., Behnke, A. R., Ann. N.Y. Acad. Sci., 1963, v-110, 1-1018.

4. Croxton, F. E., Elementary Statistics and Applications in Medicine and the Biological Sciences, 1959, Dover, New York.

5. Depocas, F., Hart, J. S., J. Appl. Physiol., 1957, v10, 388.

6. Huxley, J. S., Problems of Relative Growth, 1932, Methuen and Co., London.

7. Joy, R. J. T., Mayer, J., J. Appl. Physiol., 1967 (in press).

8. Kleiber, M., The Fire of Life, 1961, John Wiley and Sons, New York.

9. Mayer, J., Vitale, J. J., Am. J. Physiol., 1957, v189, 39.

10. Miller, I., Weil, W. B., Jr., Ann. N.Y. Acad. Sci., 1963, v110, 153.

11. Rathbun, E. N., Pace, N., J. Biol. Chem., 1945, v158, 667.

12. Therriault, D. G., Poe, R. H., Canad. J. Biochem., 1965, v43, 1427.

13. von Döbeln, W., Acta Physiol. Scandinav., 1956, v37, Suppl. 126.

14. Zucker, L., Zucker, T. F., J. Gen Physiol., 1941, v25, 445.

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

Further Characterization of Specific Heterophile Antibody from Infectious Mononucleosis Serum.* (32592)

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The biochemical and immunological characteristics of immunoglobulins belonging to the IgM class have been reported for certain disease conditions, such as multiple myeloma, chronic hemolytic anemia and Waldenstrom's macroglobulinemia(1-8). In a previous contribution to this journal(9) we reported on the isolation and purification of the specific heterophile antibody from infectious mononucleosis. In the present report, further observations on the characterization of the heterophile antibody with particular emphasis on differences between the IgM of the heterophile antibody and non-heterophile antibody IgM are presented.

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* Supported by Grants AI-06157 and 3-T01-AI 00304 from Nat. Inst. Health, USPHS.

‡ André Nahmias is recipient of a Research Career Development Award from the USPHS.

Materials and methods. Isolation of heterophile antibody (HA). As previously described(9) HA was isolated by adsorption with beef cell antigen of serum from patients with infectious mononucleosis; this serum had been submitted to prior adsorption with guinea pig kidney antigen. The heterophile antibody was dissociated from the beef cell antigen by an ether elution method and was further purified by sucrose density gradient ultracentrifugation. The purified concentrated eluates, when tested by the double diffusion agar method and immunoelectrophoresis, were noted to give only 1 line with goat antihuman IgM serum.

Electrophoresis of purified HA. The purified eluate was tested by cellulose acetate strip electrophoresis using LKB apparatus. Cellulose acetate strips (S & S, Serometrics, Inc.) of 18 × 2.4 cm size were used with the Tris (80.0 g/l), boric acid (8.0 g/l), EDTA (6.0

g/l), sodium azide (8.0 g/l) buffer recommended by Aronsson and Gronwall(10). Electrophoresis was run at 0.75 mA/cm and 150 volt at room temperature for 3 hours. Strips were fixed with 200 g/l sulfosalicylic acid in water and stained with 2.5 g/l Coomassie brilliant blue R250 in glass distilled water as recommended by Fazekas de St. Groth *et al* (11).

Preparation of rabbit anti-HA serum. 3 ml of purified heterophile antibody which had a 1:4000 sheep agglutination titer were injected intravenously into a rabbit twice a week for 4 consecutive weeks. 10 days after final immunization, the activity of the rabbit serum was tested by the double diffusion agar method. The precipitin titer of this immune rabbit serum was 1:8 against a purified HA which had a sheep erythrocyte agglutination titer of 1:4000.

Preparation of non-HA IgM. Heterophile antibody from infectious mononucleosis serum was first adsorbed completely with beef cell antigen. 2 ml of this serum were applied to Sephadex G-200 column chromatography. IgM containing fractions were pooled and were concentrated by ultrafiltration. This concentrated material was then submitted to sucrose density gradient ultracentrifugation. IgM containing fractions were pooled and concentrated by ultrafiltration. This final sample did not show any sheep erythrocyte agglutination activity and reacted only with goat antihuman IgM serum (Hyland Labs).

Examination of L chains. Anti-lambda and anti-kappa goat sera (obtained from Hyland Labs) were used for these experiments. The original serum from patients with infectious mononucleosis was tested at a 1:8 dilution. The purified heterophile IgM and non-heterophile IgM were used undiluted. All preparations were tested by the double diffusion agar method against anti-lambda and anti-kappa serum, as well as against anti-IgM, anti-IgA, anti-IgG and antihuman goat sera.

Examination for inhibitory activity of infectious mononucleosis patient's sera by anti-HA rabbit serum. Sera with sheep erythrocyte agglutination titers ranging from 1:320 to 1:20,480 were obtained from 10 patients with infectious mononucleosis. Heterogenous sheep erythrocyte agglutinins of the immune anti-HA rabbit serum were adsorbed with sheep red cells prior to use. 0.1 ml of a 1:10 dilution of serum from each patient was then diluted 1:2 to 1:8182 with phosphate buffer saline (1/15 M pH 7.0—PBS) and 0.1 ml of 1:5 anti-HA rabbit serum was added to each tube. As a control, PBS was used in place of the immune rabbit serum. The mixtures were incubated at 37°C for one hour; 0.1 ml of a 1.25% sheep erythrocyte suspension was added to each tube, incubated at 37°C for 30 minutes and at 4°C for 4 hours and the agglutination titers read.

Results. Electrophoresis of purified HA. When tested by cellulose acetate membrane electrophoresis, purified HA produced only 1 band located in the slow gamma globulin area as compared to whole serum from patients with infectious mononucleosis (Fig. 1).

L chains of purified HA. Using the Ouchterlony immunodiffusion technique the serum (Fig. 2—top) and the non-HA IgM (Fig. 2—middle) from a patient with infectious mononucleosis showed both kappa and lambda types of the L chain. However, purified HA showed a precipitin line against the anti-lambda type of L-chain antiserum, but no line against the anti-kappa type of L chain antiserum (Fig. 2—bottom). Similar results were obtained with serum, non-HA IgM and purified HA IgM from 3 other patients with infectious mononucleosis.

Precipitin lines between HA and non-HA IgM to anti-IgM goat serum and anti-HA rabbit serum. Using the Ouchterlony immunodiffusion technique, it was found that purified HA IgM and non-HA IgM produced precipitin lines of identity against anti-IgM goat serum (Fig. 3—left). However, HA IgM produced a line of partial identity with non-HA IgM when tested against anti-HA rabbit serum (Fig. 3—right).

Effect of anti-HA rabbit serum on sheep erythrocyte agglutinating activity of patients with infectious mononucleosis. In Table I, the effect of anti-HA rabbit serum and phosphate buffered saline on the heterophile activity of serum from 10 patients with infectious mononucleosis is presented. The change in the number of 2-fold dilutions was found to be

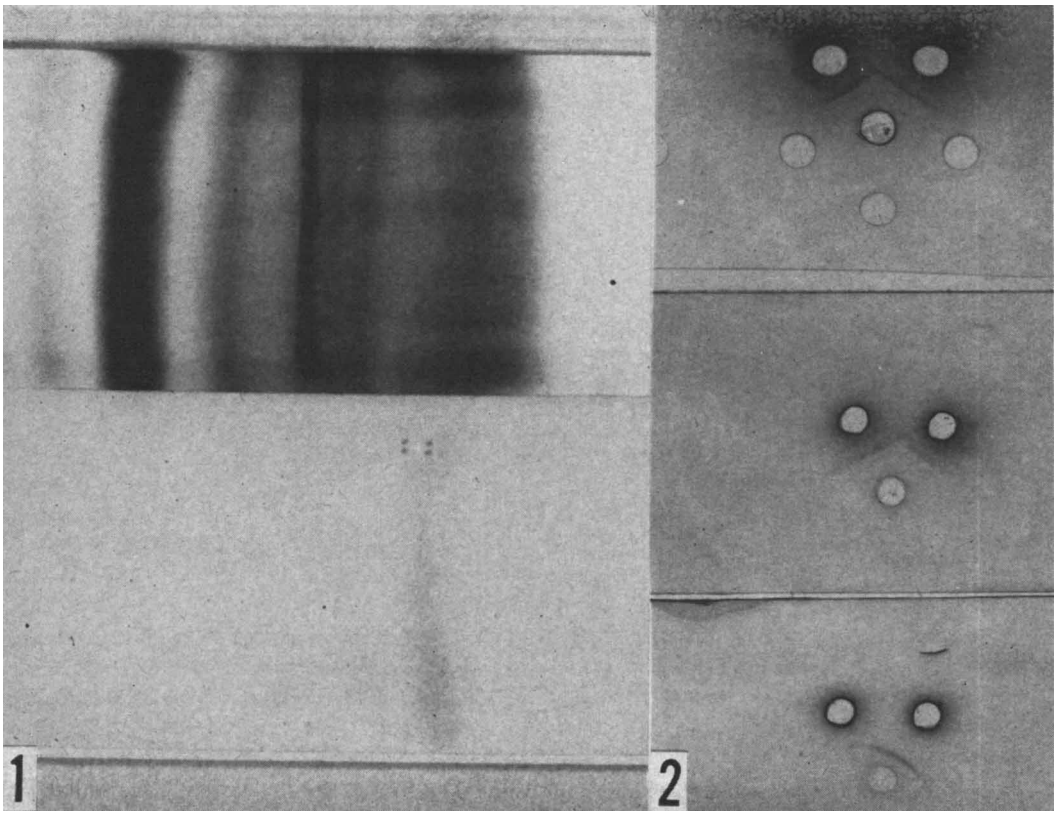

FIG. 1. Cellulose acetate membrane electrophoresis—Coomassie brilliant blue stain (top), serum of patient with infectious mononucleosis; (bottom), purified heterophile antibody from the same patient.

FIG. 2. Ouchterlony immunodiffusion test—amido black stain: In top, middle and bottom figures, upper left well contains antiserum to kappa chains, upper right well contains antiserum to lambda chains. Center well (top figure) contains serum from patient with infectious mononucleosis; center well (middle figure) contains non-herterophile antibody IgM from the same patient; center well (bottom figure) contains purified heterophile antibody IgM from the same patient.

2 in one case, 3 in 7 cases and 4 in 2 cases.

Discussion. It has been noted by several investigators that a variety of macroglobulins may develop in patients with infectious mononucleosis (IM) besides the heterophile antibody (HA), such as cold hemagglutinins, cryoglobulins and "syphilitic" antibodies(12). Wollheim and Williams(13) have also presented evidence that HA constitutes only a small percentage of the increased macroglobulins found in the serum of patients with IM. In addition, Costea *et al*(14) reported that the cold agglutinins produced in the serum of such patients possess both kappa and lambda types of the L chain.

Only a line of partial identity was found between the HA IgM and non-HA IgM when tested against anti-HA rabbit serum (Fig. 3). It has also been found that in at least 4 sera of patients with IM, only the lambda type

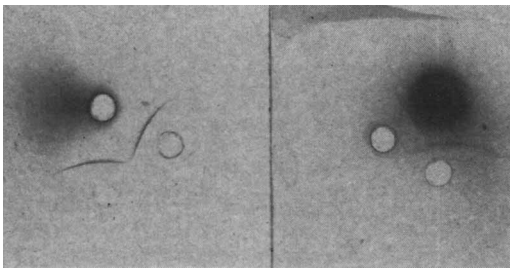

FIG. 3. Ouchterlony immunodiffusion test—amido black stain; (left) top well: anti-IgM goat serum; bottom left well: purified HA IgM; bottom right well: non-HA IgM. (Right) top well: anti-HA rabbit serum; bottom left well: purified HA IgM: bottom right well: non-HA IgM.

TABLE I. Effect of Anti-Heterophile Rabbit Serum on the Sheep Erythrocyte Agglutinating Activity of Serum of Patients with Infectious Mononucleosis.

Infectious mononucleosis serum	Sheep erythrocyte agglutinating titer in presence of: Phosphate buffer saline	Anti-HA serum	No. of 2-fold dilutions change
1.	1:32	1:4	3
2.	1:1024	1:128	3
3.	1:1024	1:256	2
4.	1:2048	1:256	3
5.	1:1024	1:128	3
6.	1:128	1:16	3
7.	1:2048	1:128	4
8.	1:1024	1:64	4
9.	1:2048	1:256	3
10.	1:2048	1:256	3

of the L chain was demonstrable, while the non-HA IgM fractions from sera of the same patients possessed both kappa and lambda types (Fig. 2).

In view of findings with other macroglobulins, there is no assurance that this observation will be universal with all infectious mononucleosis heterophile anitbodies. For instance, the IgM from cases of Waltenstrom's macroglobulinemia, has been found by Fahey and Solomon(3) to contain only type kappa in 18 instances and type lambda in 4 instances. Also, while the IgM of cold agglutinins from 59 patients with chronic hemolytic anemia has been found to contain only type kappa(6), more recently type lambda only has been noted in sera from 3 patients with this condition(15).

It has been noted by several observers that children under the age of 5 years(16) and certain adults(17) have negative heterophile antibody tests with a clinical picture compatible with infectious mononucleosis. It has been proposed(16) that either these individuals did not actually have this disease or that they were unable to respond immunologically. In view of the differences noted by several workers and ourselves among the various macroglobulins elicited in infectious mononucleosis, 1 possible explanation would be that the non-responsive individuals lack the clones of cells involved in HA synthesis or that these particular clones do not respond to the antigenic stimuli. Certain similarities exist between infectious mononucleosis and *Mycoplasma pneumoniae* infections, since in the latter condition the cold agglutinin (a macroglobulin) response is present in only about 50% of cases(18).

MacKenzie and Deutsch(19), using rhesus monkey antisera to individual Rh saline antibodies and to Waldenstrom IgM globulins, demonstrated serological differences between IgM antibodies such as Rh saline agglutinins, the α and β iso-hemagglutinins and cold and heterophile agglutinins. We attempted to use a similar approach by testing the ability of anti-HA rabbit serum to reduce the sheep erythrocyte agglutinating titer of sera from 10 patients with IM (Table I). We could not show any marked differences among the 10 sera, while Wollheim and Williams(13), using antisera of monoclonal IgM were able to show varying anti-hemagglutinating effects on the heterophile antibody from different patients. In view of the differences in approaches and findings between these workers and ourselves, the question of the antigenic heterogeneity among IgM molecules and heterophile antibody from infectious mononucleosis sera remains open to further investigation. Also of interest for further study are the heterophile antibodies which have different guinea pig and beef cell adsorption patterns than those of infectious mononucleosis. Such studies would present a clearer understanding of the origin and characteristics of these macroglobulins.

Summary. Specific heterophile antibody prepared by adsorption to beef erythrocytes and elution with diethyl ether was further characterized. When tested by cellulose acetate membrane electrophoresis the purified preparation produced one band in the slow gamma region. Whereas the whole serum and the non-heterophile antibody IgM fractions from 4 patients with infectious mononucleosis possessed both kappa and lambda types of the L chain, the heterophile antibody IgM from these patients contained only the lambda type. In addition, the IgM of the heterophile and non-heterophile fractions of such patients' sera showed a line of partial identity when tested by immunodiffusion against anti-heterophile antibody rabbit serum. There was no significant

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.

1. Mannik, M. M., Kunkel, H. J., Exp. Med., 1962, v116, 859.
2. Migita, S., Putnam, F. W., ibid., 1963, v117, 81.
3. Fahey, J. L., Solomon, A. J., J. Clin. Invest., 1963, v42, 811.
4. Gross, D., Epstein, W., ibid., 1964, v43, 83.
5. Frangione, B., Franklin, E., J. Exp. Med., 1965, v122, 1.
6. Harboe, M., Deverill, J., Scand. J. Haemat., 1964, v1, 223.
7. Harboe, M., Scand. J. Clin. Lab. Invest., (Suppl. 84) 1965, v17, 223.
8. Wollheim, F. A., Williams, R. C., Acta Med. Scand. (suppl. 445) 1966, v179, 115.
9, Yoshida, K., Nahmias, A. J., Proc Soc. Exp. Biol. & Med., 1967, v124, 515.
10. Aronsson, T., Gronwall, A., Scand. J. Clin. and Lab. Invest., 1957, v9, 338.
11. Fazekas de St. Groth, S., Webster, R. G., Datyer, A., Biochimica et Biophysica Acta, 1964, v71, 377.
12. Damashek, W., Acta Med. Scand. (Suppl. 445) 1966, v179, 163.
13. Wollheim, F. A., Williams, R. C., New Eng. J. Med., 1966, v274, 61.
14. Costea, N., Yakulis, V., Heller, P., Science, 1966, v152, 1520.
15. Feizi, T., ibid., 1967, v156, 111.
16. Valquest, B., Ekelund, H., Tucteras, E., Acta paediatrica (suppl. 114) 1958, v47, 120.
17. Leibowitz, S., Ann. Int. Med., 1956, v44, 717.
18. Chanock, R. M., Mufson, M. S., Bloom, H. H., James, W. D., Fox, H. H., Kingston, J. R., JAMA, 1961, v175, 213.
19. MacKenzie, M. R., Deutsch, H. F., J. Immunol., 1965, v95, 87.

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 absorb 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 Psycrotherm 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.