was greater in the presence of cephalin (PE) than lecithin, oleic acid than palmitic acid, and globulin than albumin.

1. Kates, M., in *Lipide Metabolism,* edited by K. Bloch, New York, John Wiley & Sons, Inc., 1960, p214.

2. Gollub, S., Schechter, D. C., Kaplan, F. E., Meranze, D. R., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 858.

3. Kushner, D. J., Feldman, D., *Biochim. et Biophys. Acta,* 1958, v30, 466.

4. Vogel, W. C., Zieve, L., *J. Lipid Research,* in press.

5. Deuel, H. R., Jr., *The Lipids, Their Chemistry and Biochemistry,* New York, Interscience Publishing Co., 1957, vIII, p869.

6. Chu, H. P., *J. Gen. Microbiol.,* 1949, v3, 255.

Received October 4, 1963. P.S.E.B.M., 1963, v114.

Strain Specific Complement-Fixation Differentiation of Influenza B Viruses Isolated in Tissue Culture.* (28805)

AKINYELE FABIYI, CLIFTON R. GRAVELLE AND TOM D. Y. CHIN
(Introduced by H. A. Wenner)
Section of Virus Research, Department of Pediatrics, and Department of Microbiology, University of Kansas School of Medicine, and Kansas City Field Station, Communicable Disease Center, Public Health Service, U. S. Department of HEW, Kansas City, Kansas

The nature and relationships to each other of the two types of complement-fixing (CF) antigens (soluble and viral) of influenza viruses have been well established(1-5). With the development of strain specific and type specific antisera and antigens(5-7) it has been possible to apply the CF method in serodiagnosis and epidemiologic studies of influenza infections(8,9). In these studies, however, emphasis was placed on influenza type A strains, propagated or isolated in the developing chick embryo.

Many influenza B strains isolated during the 1961-62 epidemics in the Midwest of the United States did not propagate in the chick embryo, but were propagated in monkey kidney tissue culture (MKTC). We have been able to apply the strain-specific CF test and the hemagglutination-inhibition (HI) primarily to study antigenic variations of the isolated strains by using acute and convalescent sera from influenza B epidemics in this area. The results of the analysis are reported here.

Materials and methods. Tissue culture (TC) cells cultivated from kidneys of rhesus *(Macaca mulatta)* monkeys (MKTC) were used for virus isolation. The cells were maintained in a modified Eagle's medium with high cystine as described previously(10).

Virus strains. Influenza B virus strains isolated in MKTC from oropharyngeal secretions of patients with influenza were selected for serologic studies. Strain Illinois 61-952 came from Grand Tower in southern Illinois, strain Iowa 62-21 from Hazelton in northeast Iowa, strain Missouri 61-482 from Potosi, Mo., strain Missouri 61-967 from Jefferson City, Mo., and strain Missouri 62-36 from Marshall, Mo. Jefferson City and Marshall are located in central Missouri, separated by a distance of about 70 miles. Potosi is about 65 miles southwest of St. Louis. All strains were isolated from influenza cases during the winter of 1961-1962. Each strain has had less than 5 passages in MKTC.

Sera. Acute and convalescent sera were obtained from 8 cases of influenza: 1 from Kansas City, Mo., and 7 from Potosi in southeast Mo. Influenza B virus was isolated from the oropharyngeal secretions of the 8 cases. None of the cases had given a history of recent influenza vaccination.

Complement-fixation (CF) test. The method used has been described(11). Sera were inactivated at 56°C for 30 minutes before use.

Hemagglutination (HA) and hemaggluti-

* This investigation was supported in part by U. S. P. H. S. research grant from Nat. Cancer Inst.

TABLE I. Titers* of CF Test with Human Acute and Convalescent Sera and Influenza B Virus Strains Isolated in MKTC.

Case No.	Age (yr)	Days from onset of illness	Influenza antigens: Illinois 61–952	Iowa 62–21	Missouri§ 61–482	Missouri 61–967	Missouri 62–36
1†	12	1/28‡	0/128	0/ 512	ND	0/128	0/ 512
2	12	2/18	0/128	0/ 128	0/128	32/256	32/ 512
3	13	2/18	8/256	8/ 512	8/ 64	32/512	32/>512
4	23	3/19	8/ 32	0/ 512	8/ 32	16/ 32	32/ 8
5	26	5/21	0/512	0/>512	0/ 32	8/ 32	0/ 0
6	27	1/17	0/512	8/>512	ND	32/512	32/ 512
7	30	1/19	0/512	0/ 512	0/ 8	8/512	8/ 512
8	45	6/22	0/ 64	0/ 512	0/ 0	32/128	32/ 256

* Reciprocals of dilutions. † The only case not from Potosi, Mo. ‡ Numerator, acute; denominator, convalescent. § See text.
0 = less than 1:8. ND = not done.

nation-inhibition (HI) tests modified by incubation of virus-serum mixture at 4°C overnight were performed as described by Hilleman *et al*(12). CF and HI tests with strains Illinois 61-952, Iowa 62-21, Missouri 61-967 and Missouri 62-36 were carried out at the same time; tests with strain Missouri 61-482 were done later. This strain, Missouri 61-482, was highly unstable.

Preparation of HI and CF antigens followed essentially the method of Lief and Henle(6) but modified by low speed centrifugation (1500 rpm for 20 minutes) of infected MKTC, previously frozen and thawed twice, to remove cellular debris. The supernatant was then centrifuged in the high speed ultracentrifuge (Spinco model L with 40 head) at 40,000 rpm for 2 hours. Pellets were resuspended to 1/10 of original volume in phosphate buffered saline (PBS)(6). Two cycles of adsorption to human "O" erythrocytes and elution at 37°C for 2½ hours with 1% receptor destroying enzyme in PBS(6) followed. The final concentration of each antigen was 10 × that of the original.

Results. Results of CF tests with acute and convalescent sera and 5 strains of influenza B virus isolated in MKTC are shown in Table I. A 4-fold or greater rise of antibody titer with paired sera was demonstrated in sera of all 8 patients using Illinois 61-952 and Iowa 62-21. Antibody rise could not be demonstrated in the sera of one patient (case 4) using strain Missouri 61-967 as antigen, or in sera of 2 patients (cases 4 and 5) using strain Missouri 62-36 as antigen. Of the 6 paired sera tested with Missouri 61-482 antigen, 4 showed 4-fold or greater rises, one showed a rise but of low titer (1:8) in the convalescent serum and one had no detectable antibody in either the acute or convalescent serum.

The reactions of the sera of case 1 (the only one not from Potosi, Mo.) and cases 5 and 7 were similar, in that the antibody titers of the acute sera were either low, or undetectable to all the antigens tested. In the remaining 5 cases, however, the reactions of the paired sera to these antigens were variable, particularly in case 4. In these 5 the CF titers to the Illinois and Iowa strains were similar, but they were different from those obtained with Missouri strains 61-967 and 62-36 which are closely related antigenically. The antibody pattern obtained with strain Missouri 61-482 indicated that this virus was somewhat different from other strains.

The results of HI tests with the same sera and influenza B virus strains used in the CF test are shown in Table II. A 4-fold or greater rise of HI antibody titers was demonstrated with 3 of the influenza B strains for paired sera of all 7 patients; with the other 2 strains, 4-fold rises could not be demonstrated for 2 patients. Unlike the CF test, there was little variation in HI titers to the 5 influenza virus strains.

Discussion. From the CF antibody pattern, it appears that strains Illinois 61-952 and Iowa 62-21 are closely related and are antigenically somewhat different from the 2

TABLE II. Titers* of HI Test with Human Acute and Convalescent Sera and Influenza B Virus Strains Isolated in MKTC.

Case No.	Age (yr)	Days from onset of illness	Influenza antigens: Illinois 61–952	Iowa 62–21	Missouri‡ 61–482	Missouri 61–967	Missouri 62–36
2	12	2/18†	960/ 3840	240/3840	ND	960/ 960	240/3840
3	13	2/18	960/ 3840	240/3840	ND	240/ 3840	240/3840
4	23	3/19	960/ 3840	240/ 960	160/ 160	60/ 240	240/ 960
5	26	5/21	960/ 3840	240/3840	160/1280	60/ 960	240/3840
6	27	1/17	960/ 3840	240/3840	ND	960/ 960	240/3840
7	30	1/19	960/ 3840	240/3840	40/ 320	60/>3840	240/3840
8	45	6/22	960/>3840	240/3840	160/ 320	240/ 960	240/ 960

* Reciprocals of dilutions. † Numerator, acute; denominator, convalescent. ‡ See text. ND = not done.

closely related Missouri 61-967 and Missouri 62-36 strains; the Missouri 61-482 strain may be considered to be intermediate between the two groups. By using either the Illinois 61-952 or the Iowa 62-21 strains as CF antigens, antibodies were undetectable in the acute phase sera of 6 patients, while a titer of 1:8 was shown by 2 patients. Of 6 paired sera tested against the Missouri 61-482 strain, antibodies were undetectable in acute sera of 4 patients similar to the Iowa-Illinois group reaction. Two of the 6 showed titers of 1:8, a reaction similar to that obtained with the antigenically closely related 2 Missouri strains. The CF titers in the convalescent sera also varied, but to a lesser extent, than for the 4 other influenza B strains. The Missouri 482 strain, however, appeared to be a poor antigen as shown by the low antibody levels detected in the convalescent sera by both CF and HI tests. In contrast, antibody levels of from 1:8 to 1:32 were detectable in the acute phase sera of 7 patients using the other Missouri strains.

Neither CF nor HI antibodies could be detected in the acute and convalescent sera of case 8 with Missouri 61-482 strain, and antibodies were not demonstrable in both sera of case 5 against Missouri strain 62-36. The 61-482 strain was isolated from case 5. Despite the absence of significant CF and HI antibodies in these sera to the influenza strains mentioned serologic confirmation of influenza B infection was established by the demonstration of 4-fold or greater antibody rises against the other virus strains.

These results indicate that the CF test could detect minor antigenic differences among intratypic strains of influenza virus isolated from different localities in the same season. The CF test could also detect differences among strains isolated from the same locale (Missouri). These differences were not demonstrable by the HI tests because of the broad antibody spectra shown. Since none of the cases had recent influenza vaccinations, the high HI titers cannot be attributed to vaccine induced antibodies, but probably represent an amnestic response of infection.

Strain-specific guinea pig antisera(5) have been used in the CF test to demonstrate antigenic variations among influenza virus isolates. The results reported here show that human sera can also be useful in the CF test to demonstrate strain differences among influenza B virus strains isolated in MKTC.

Summary. Five strains of influenza B virus isolated in monkey kidney tissue cultures from 5 separate influenza outbreaks in the Midwest of the United States during the winter of 1961-62 were studied for antigenic variations. These strains were analyzed by the strain specific CF test and the standard HI test. The sera used were obtained during the acute and convalescent phases from 8 patients who had influenza B infection confirmed by virus isolation. The CF test could detect antigenic differences among influenza strains isolated from the same locale as well as from different geographic areas. These differences were not detectable by the HI test.

The technical assistance of Mrs. Evelyn Welton,

Mr. John Blize, and Miss Jacqueline Ashby is greatly appreciated. We thank Dr. H. A. Wenner for advice and encouragement.

1. Lennette, E. H., Horsfall, F. L., Jr., *J. Exp. Med.*, 1941, v73, 581.
2. Henle, W., Wiener, M., PROC. SOC. EXP. BIOL. AND MED., 1944, v57, 176.
3. Hoyle, L., *J. Hyg.*, 1945, v44, 170.
4. Lief, F. S., Fabiyi, A., Henle, W., *J. Immun.*, 1958, v80, 53.
5. Fabiyi, A., Lief, F. S., Henle, W., *J. Immun.*, 1958, v81, 467.
6. Lief, F. S., Henle, W., *Bull. WHO,* 1959, v20, 411.
7. Lief, F. S., *J. Immun.*, 1963, v90, 172.
8. Henle, W., Lief, F. S., Fabiyi, A., *Lancet,* 1958, v1, 814.
9. Lief, F. S., Ostapiak, M., Fabiyi, A., Henle, W., *J. Immun.*, 1958, v81, 478.
10. Dubes, G. R., Chapin, M., *J. Gen. Microbiol.*, 1958, v18, 320.
11. Fabiyi, A., Wenner, H. A., PROC. SOC. EXP. BIOL. AND MED., 1963, v113, 81.
12. Hilleman, M. R., Mason, R. P., Buescher, E. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 829.

Received October 7, 1963. P.S.E.B.M., 1963, v114.

Influence of Acid Polysaccharides on Plaque Formation by Influenza A2 and B Viruses. (28806)

K. K. TAKEMOTO AND P. FABISCH (Introduced by S. Baron)
U. S. Department of HEW, Public Health Service, Nat. Inst. Health, National Institute of Allergy and Infectious Diseases, Laboratory of Biology of Viruses, Bethesda, Md.

Although a number of investigators have reported plaque production by certain strains of influenza viruses(1-6) plaque assays with these viruses in general have not been very successful. Simpson and Hirst(5) for example, examined a number of influenza A and B strains for ability to form plaques and most of these were either negative or formed faint plaques. It was recently reported from this laboratory(7) that plaque development and morphology for EMC virus was markedly influenced by the sulfated polysaccharide which is an inherent component of the agar used in most procedures involving the plaque technique. It has subsequently been shown that this polyanion is active against a number of different animal viruses(8,9,10). The studies reported here were undertaken to determine the influence of sulfated polysaccharides on plaque production by a strain of influenza A2 and a recently isolated B strain, utilizing the procedure for removal of the inhibitor with polycations(11).

Materials and methods. Viruses. The influenza A2 and B strains used in this study were isolated by Dr. Robert M. Chanock in rhesus monkey kidney cultures (MK) and were received as the third passage in these cells, without cultivation in other hosts. Up to 4 additional passages were made in this laboratory in MK cultures and these virus stocks were used throughout these experiments. Virus titers ranged from $10^{5.5}$ to 10^{7} pfu/ml when plated under conditions described below.

Cell cultures and media. MK cultures were prepared by a modification of Youngner's trypsinization procedure(12) and grown in 60 mm Petri plates in a medium of Earle's salt solution, 5% fetal bovine serum, and 0.5% lactalbumin hydrolysate. The cultures were grown in a humidified incubator containing 4% CO_2. The overlay medium for plaque assays consisted of the following: 0.1% yeast extract (Difco), 0.1% bovine albumin (Armour Pharmaceutical Co.), Earle's salt solution, 1% fetal bovine serum, and 0.9% Noble agar (Difco). DEAE-dextran (Pharmacia, Uppsala, Sweden) was dissolved in sterile distilled water at a concentration of 10 mg/ml and the desired concentrations were added to melted 1.8% agar at 44°C. An equal volume of 2 × concentrated nutrient medium was then added to the agar solution prior to pouring into inoculated plates. For staining of cultures, the same