Antigenic Relationship Between Echovirus Types 29 and 32.* (29164)

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The Panel for Picornaviruses has recently recognized 4 new echoviruses, types 29-32 (1). The prototype of type 29 is the JV-10 strain, the Bastianni strain is the prototype of type 30, the Caldwell strain serves as the prototype of type 31, and type 32 is represented by the PR-10 strain. Studies in this laboratory have shown that an antigenic relationship exists between 2 of these new echovirus immunotypes, *viz.*, types 29 and 32.

Materials and methods. Virus strains. The prototype strains of echovirus types 29 (JV-10) and 32 (PR-10), as well as the JV-6 and Taylor strains of echovirus type 29 were obtained from Dr. H. A. Wenner; subpassages were made in this laboratory, and a portion of each lot originally received was stored for reference purposes. Reference type 32 echovirus was also received from Dr. J. L. Melnick.

Immune sera. Immune serum to each echovirus type was prepared in this laboratory in rhesus monkeys(2). Reference immune sera for echovirus types 29 and 32 were received from Dr. Wenner. These were prepared under the auspices of the Board for Virus Reference Reagents, National Institute of Allergy and Infectious Diseases, N.I.H., Bethesda, Md.

Neutralization tests. For one phase of these studies, neutralizing antibody assays were conducted in tube cultures of rhesus monkey kidney cells; 2-fold dilutions of each serum were tested against approximately 100 TCD_{50} of virus, and serum neutralization endpoints were read after 6-7 days' incubation of the test cultures. Plaque reduction neutralization tests were performed on monkey kidney cell monolayers in stoppered 3 oz prescription bottles. Serum dilutions were tested against approximately 100 plaque forming units of virus, and a reduction in plaque counts of 80% or more was considered significant.

Hemagglutination-inhibition (HI) tests. Antigens were prepared in monkey kidney cell cultures, and the tests were conducted by procedures previously described(3). Prior to their examination in HI tests, monkey immune sera were absorbed with human group O erythrocytes to remove natural agglutinins and with kaolin to remove non-specific inhibitors.

Complement fixation (CF) tests. Antigens were produced in HeLa cell cultures(4), and block titrations were performed against each immune serum by the standard procedure employed in this laboratory(4).

Results. Cross neutralization tests. Originally, we had noted that our echovirus type 29 isolates were neutralized by our echovirus type 32 immune serum, the neutralizing titers ranging from 1:16 to 1:128. To determine whether there had been a mix-up in the viruses, cross neutralization tests were performed in monkey kidney tube cultures using our laboratory stock virus lots, one of our echovirus type 29 isolates, the virus lots originally supplied by Dr. Wenner under the enterovirus testing program and an echovirus type 32 reference lot supplied by Dr. J. L. Melnick. These were tested against immune sera prepared in this laboratory and against reference immune sera produced in Dr. Wenner's laboratory. Results of these tests are presented in Table I. All of the lots of echovirus type 29 were neutralized to an appreciable degree by the 2 echovirus type 32 immune sera, but the echovirus type 29 immune sera had little or no demonstrable neutralizing capacity for the echovirus type 32 lots.

Cross neutralization tests were then conducted by the plaque reduction technic, employing stock virus lots prepared in this laboratory and the reference echovirus type 32 lot from Dr. Melnick. These were tested

* The work on which this paper is based was supported by contract and grants from Nat. Inst. of Allergy and Infect. Dis., Nat. Inst. Health, U.S.P.H.S.

TABLE I. Results of Cross Neutralization Tests, Conventional Tube Technic, with Echovirus Types 29 and 32.

Virus type	Strain	Neutralizing titer of immune serum to: E29 JV-10 (CSDPH*)	E29 JV-10 (Wenner)	E29 JV-6 (CSDPH)	E29 Taylor (CSDPH)	E32 PR-10 (CSDPH)	E32 PR-10 (Wenner)
E29	JV-10	4096†	≥4096	256	256	128	32
E29	JV-10 (Wenner‡)	2048	≥4096	128	1024	64	16
E29	Hayes (isolate, plaque purified)	2048	≥1024		≥2048	64	32
E32	PR-10	<16	16	<8	<8	4096	1024
E32	PR-10 (Wenner‡)	< 8	< 8	<8	<8	4096	4096
E32	PR-10 (Melnick‡)	< 8	< 8	<8	<8	4096	2048

* Immune serum prepared by California State Dept. of Public Health, Viral & Rickettsial Disease Lab.
† Reciprocal of serum titer.
‡ Virus lot originally received from investigator was employed for tests.

against echovirus type 29 and 32 immune sera from Dr. Wenner's laboratory. In Table II it is seen that the echovirus type 29 immune serum, which showed little or no neutralizing capacity for the type 32 virus in conventional neutralization tests, had a high neutralizing antibody titer for this viral heterotype when assayed by the plaque reduction technic. In plaque reduction neutralization tests the echovirus type 32 immune serum had a titer of 1:2048 for the homotypic virus and 1:128 for echovirus type 29.

Cross hemagglutination-inhibition (HI) tests. Hemagglutinating antigens were prepared from our stock lots of echovirus type 29 and type 32 and also by subpassage of the echovirus type 32 lots originally received from Drs. Wenner and Melnick. Results of cross HI tests with type 29 and type 32 immune sera are given in Table III, and it is seen that the 2 viral types were virtually indistinguishable by this test procedure. All of the type 29 immune sera examined had high HI titers for the type 32 virus, in some cases as high or higher than those for the homotypic virus. Although the reference type 32 immune serum had a fairly high titer of homotypic neutralizing antibody, it had a low titer of homotypic HI antibody; HI titers of this serum for viral types 29 and 32 were, however, almost identical. The type 32 immune serum produced in this laboratory had a somewhat higher homotypic HI titer, but similarly its titer for type 29 virus was almost as high.

TABLE II. Results of Cross Neutralization Tests by Plaque Reduction Technic with Echovirus Types 29 and 32.

Virus type	Strain	Neutralizing titer of immune serum to: E29 JV-10 (Wenner)	E32 PR-10 (Wenner)
E29	JV-10	65,536*	128
E32	PR-10	16,384	2048
E32	PR-10 (Melnick†)	8,192	—‡

* Reciprocal of serum titer.
† Virus lot originally received from investigator was employed for test.
‡ — = Not tested.

Cross complement fixation (CF) tests. The antigenic relationship between echovirus types 29 and 32 was further indicated by cross CF tests, the results of which are shown in Table IV. Immune sera to all 3 strains of echovirus type 29 (JV-10, JV-6 and Taylor strains) fixed complement to high serum titers with the echovirus type 32 antigen. Further, the echovirus type 32 immune serum prepared in this laboratory had a CF antibody titer of 1:128 with both the type 29 and the type 32 antigens. The type 32 immune serum produced in Dr. Wenner's laboratory fixed complement non-specifically with uninfected control antigen at a dilution of 1:64, and specific fixation of complement with the type 29 antigen could not be demonstrated at serum dilutions higher than this.

Discussion. Investigators in the picornavirus field should be cognizant of the antigenic relationship between the new echovirus

TABLE III. Results of Cross Hemagglutination-Inhibition Tests with Echovirus Types 29 and 32.

Virus type	Strain	Hemagglutination-inhibiting titer of immune serum to: E29 JV-10 (CSDPH*)	E29 JV-10 (Wenner)	E29 JV-6 (CSDPH)	E29 Taylor (CSDPH)	E32 PR-10 (CSDPH)	E32 PR-10 (Wenner)
E29	JV-10	1024†	16,384	1024	256	512	16
E32	PR-10	128	4,096	4096	1024	2048	32
E32	PR-10 (Wenner‡)	128	8,192	2048	256	1024	16
E32	PR-10 (Melnick‡)	128	4,096	2048	256	1024	16

* Immune serum prepared by California State Dept. of Public Health, Viral & Rickettsial Disease Lab.

† Reciprocal of serum titer.

‡ Prepared by one subpassage of virus lot originally received from investigator.

TABLE IV. Results of Cross Complement Fixation Tests with Echovirus Types 29 and 32.

Antigen type	Strain	Complement-fixing antibody titer of immune serum to: E29 JV-10 (CSDPH*)	E29 JV-10 (Wenner)	E29 JV-6 (CSDPH)	E29 Taylor (CSDPH)	E32 PR-10 (CSDPH)	E32 PR-10 (Wenner)
E29	JV-10	128†	1024	512	1024	128	×‡
E29	JV-6	—§	—	512	—	—	—
E29	Taylor	—	—	—	1024	—	—
E32	PR-10	128	128	128	512	128	256

* Immune serum prepared by California State Dept. of Public Health, Viral & Rickettsial Disease Lab.

† Reciprocal of serum titer.

‡ Test unsatisfactory, serum reacted with uninfected control antigen at a serum dilution of 1:64.

§ Not done.

types 29 and 32. Although the degree of crossing seen in conventional tube neutralization tests was relatively minor, it could lead to confusion in the identification of viral isolates. The Committee on Enteroviruses(5,6) has recommended that 20 units of antiserum be employed in identification of viral isolates, and under these conditions the neutralizing capacity of echovirus type 32 immune serum for type 29 strains might not be apparent. However, it is recognized that higher concentrations of antiserum may be required for neutralization of "prime" strains of certain serotypes(5,7,8,9), and in actual practice immune sera are usually employed for identification of isolates at dilutions containing more than 20 units of antibody. If neutralization of viral isolates is observed with both echovirus type 29 and type 32 immune sera, definitive identification should be undertaken by testing a standard dose of the virus against serial dilutions of each immune serum. The studies described herein show that HI and CF tests are unsatisfactory for distinguishing between echovirus types 29 and 32.

The demonstration of an antigenic relationship between echovirus types 29 and 32 raises the question as to whether these two viruses should actually be considered distinct immunotypes. They can not be distinguished from each other by HI and CF tests, as recommended by the Committee on Enteroviruses(6). The cross reactivity observed in conventional tube neutralization tests was relatively minor, but that seen in plaque reduction tests leaves little doubt as to the strong degree of antigenic relationship between these two new viral types. Other investigators(9) have reported evidence of antigenic variation among recognized strains of echovirus type 29; immune serum to the JV-6 strain of this immunotype, which had a homologous neutralizing antibody titer of 1:8192, neutralized the Taylor strain of echovirus type 29 to a serum titer of only 1:25. It might be more appropriate to consider the PR-10 strain an

intratypic variant of echovirus type 29 rather than a distinct echovirus serotype.

Summary. An antigenic relationship has been demonstrated between the newly classified echovirus types 29 and 32. The amount of crossing seen in conventional tube neutralization tests was relatively minor, but fairly high reciprocal titers were observed in plaque reduction neutralization tests. In cross hemagglutination-inhibition and complement fixation tests these two viral types were virtually indistinguishable. The question arises as to whether these two new echovirus types should be classified as distinct immunotypes or whether they represent antigenic variants of a single viral type.

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Received January 28, 1964. P.S.E.B.M., 1964, v116.

Comparison of Bactericidal and Hemolytic Serum Systems. I. Inhibitory Effects of Normal Human Serum Fractions.* (29165)

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Factors responsible for the bactericidal activity of sera *in vitro* have been investigated extensively(1-5) and it has been shown that they involve specific antibody and the known components of the hemolytic complement system. In addition, lysozyme has been implicated as a potentiating factor of the reaction(6). Earlier studies(7) in this laboratory have shown that the bactericidal serum reaction can be enhanced by certain amino acids and carbohydrates and can be inhibited by factors interfering with the normal metabolic activities of the cell. It became of interest, therefore, to determine to what extent possibly unrecognized factors in serum, when present in excess, might modify the bactericidal activity of serum. To this end, each of 32 fractions of a pool of human serum was tested for its ability to modify a basic bactericidal serum reaction. In these tests no enhancing effects but inhibitory effects were detected in the presence of an excess of certain serum fractions. Since the serum fractions responsible for such inhibitions differed from fractions previously shown to inhibit the hemolytic activity of complement (8), the analysis with the 32 serum fractions was extended to include a study of inhibitory effects on immune hemolysis, so that a comparison between effects on bactericidal and hemolytic systems could be made. The results are presented here.

Materials and methods. The bactericidal reactions were carried out by previously detailed methods(7), using pools of so-called "normal" human serum (obtained from Middlesex Hospital, New Brunswick, N. J. or Interstate Blood Bank, Inc., Memphis, Tenn.) as a source of specific antibody, complement and other accessory factors. To the reaction mixture (0.5 ml) containing 0.1 ml of "normal" serum diluted with saline (1/10, 1/20, or 1/40) and 0.2 ml of the fraction to be tested (diluted 1/4, 1/8, or 1/16), was added 0.2 ml of a suspension of *Escherichia coli* 8 in ABA buffer(9) containing 10^3 cells.

* This work was supported in part by grant from U. S. Public Health Service.