On the Alleged Antigenic Relation Between ECHO Virus Types 29 and 32.* (30333)

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A recent report(1) indicated that an antigenic relationship exists between ECHO virus types 29 and 32 and that this relationship is more apparent by plaque-reduction, hemagglutination-inhibition, and complement-fixation tests than by conventional tube neutralization tests. Further collaborative studies have shown that this conclusion was not valid because it was based on work done with a mixture of the viruses in question. This report will summarize data obtained with purified reagents in the more recent experiments which failed to detect an antigenic relationship between ECHO virus types 29 and 32.

Materials and methods. It was known that each of the ECHO type 32 virus pools employed by Schmidt *et al*(1) had been derived from a single pool of the 12th monkey kidney passage of the prototype(2) PR-10 strain. All virus pools derived from this particular source will be referred to in this report as E32 (MK12). When preliminary experiments indicated that the apparent antigenic relationship between ECHO types 29 and 32 might have resulted from a pool of the latter virus having been contaminated with the former, further tests were carried out with ECHO type 32 virus which had *not* been derived from E32 (MK12). Some of these tests were done with a virus pool derived from a new terminal dilution "purification" of the 1st monkey kidney passage of PR-10 virus and others were done with pools derived from the 11th monkey kidney passage of the same virus. Pools derived from these sources will be referred to as E32 (MK1) and E32 (MK11) respectively.

A number of different passage levels and pools of the prototype strain (JV-10) of ECHO type 29 virus were employed. Since the results with all pools were essentially the same, in the interest of brevity, passage levels and other data will not be given.

Many different antisera were employed by the various laboratories. When the data were assembled all were consistent, hence, for the sake of simplicity, results with only some of the antisera will be recorded here. Two ECHO type 29 antisera prepared in rhesus monkeys or baboons with the prototype strain will be referred to as E29 (Kansas) and E29 (Baylor) respectively. Four ECHO type 32 antisera will be referred to as follows: those prepared in rhesus monkeys with PR-10 (MK12) virus as E32 (Kansas) and E32 (California); one prepared in baboons with PR-10 (MK12) virus as E32 (Baylor); and one prepared in a rabbit with PR-10 (MK1) virus as E32 (Hawaii).

Although slightly different techniques were used by the various laboratories in performing tube neutralization, plaque-reduction, hemagglutination, and hemagglutination-inhibition (HI) tests, these variations, with one exception, did not appear to be of critical importance. One laboratory was able to obtain plaques with what was eventually determined to be "pure" PR-10 virus by incorporating 100 μg/ml of DEAE dextran in the overlay (H. Rouhandeh, personal communication) whereas two other laboratories were unable to obtain plaques with this virus using their standard techniques. Each of three laboratories obtained plaques with the ECHO type

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TABLE I. Results of Cross Neutralization, Cross Plaque-Reduction, and Cross Hemagglutination-Inhibition Tests with ECHO Virus Types 29 and 32.*

Virus type and source	Serum type and source: E29 Kansas	E29 Baylor	E32 Kansas	E32 Calif.	E32 Baylor	E32 Hawaii
			Neutralizing antibody titers†			
E29	≧4096	32768	800	64	<8	<10
	50000	>32000	>128		<8	
		16000	100		<25	
E32 (MK11)	<32	<8	>12800	4096	32768	—
		<32	6400		>32000	
		<25			>6400	
E32 (MK1)	<50	— ‡	1000	—	—	500
			Plaque-reduction antibody titers†			
E29	65536	65536	—	—	<8	—
		>6400			<25	
E32 (MK11)	—	<25	—	—	>6400	—
			Hemagglutination-inhibition antibody titers†			
E29	16384	2048	320	512	<16	<10
	5120	640	160		<20	
	5120	2560	320		10	
E32 (MK12)	4096	2048	1280	1024	<16	<10
	10240	320	1280		<20	
	2560	640	320		<10	

* Multiple results for the same virus-serum pair represent data from different laboratories.
† Reciprocal of serum dilution.
‡ — = not done.

29 virus and with "contaminated" PR-10 virus with standard techniques.

All neutralizing antibody titers were determined employing a virus dosage of between 30 and 364 $TCID_{50}$ and all HI titers were determined by using approximately 4 hemagglutinating antigen units.

Results. Cross neutralization, plaque-reduction, and hemagglutination-inhibition results with ECHO type 29 and 32 viruses and antisera are shown in Table I. It will be noted that neither of the ECHO type 29 sera neutralized E32 (MK1) or E32 (MK11). When E32 (MK12) virus was tested against the same 2 sera highly variable results were obtained ranging from no neutralization to high neutralizing titers. Since these results suggested that virus pools derived from E32 (MK12) might contain varying amounts of the 2 viruses it would be expected that antisera prepared with such pools would also contain varying amounts of antibody to each virus. It will be noted that of the 3 antisera prepared with E32 (MK12) 2 (Kansas and California) neutralized ECHO type 29 virus to some extent whereas one (Baylor) did not. The E32 Baylor antiserum had been prepared with a virus pool which had been inoculated with seed diluted 1:100 from the previous passage. Hence, it appears that this seed had inadvertently been "purified" by dilution. When only those reagents which were not in some way connected with E32 (MK12) are considered it will be noted that no cross neutralization was detected. The results of the tube neutralization tests were confirmed by the plaque-reduction tests.

Each of the laboratories participating in this study was able to obtain hemagglutination with virus pools derived from E32 (MK12) whereas none were able to do so with E32 virus not derived from this source. This hemagglutination was inhibited by each of the antisera which neutralized ECHO type 29 virus whereas it was not inhibited by those antisera which neutralized only E32 (MK11) or E32 (MK1) virus. Furthermore, a rabbit antiserum prepared from a hemagglutinating virus pool derived from E32 (MK12) which had been "purified" by a terminal dilu-

tion had a neutralizing antibody titer of <25 against E32 (MK1) and a titer of 1:1000 against its homologous seed.

Discussion and summary. The data presented here indicate clearly that the previously reported antigenic relationship between ECHO types 29 and 32 was the result of working with a mixture of the viruses in question. No antigenic relationship between these types was detected by tube neutralization, plaque-reduction, or hemagglutination-inhibition tests when "purified" reagents were employed.

1. Schmidt, N. J., Ho, H. H., King, C. J., Dennis, J., Lennette, E. H., Proc. Soc. Exp. Biol. and Med., 1964, v116, 77.

2. Branche, W. C., Jr., Young, V. M., Houston, F. M., Koontz, L.W., ibid., 1965, v118, 186.

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